Issued February 7, 1910.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN 118.

A. D. MELVIN, CHIEF OF BUREAU.

CULTURAL STUDIES OF SPECIES OF PENICILLIUM.

BY

CHARLES THOM, PH. D.,

Mycologist in Cheese Investigations, Dairy Division.

WASHINGTON:

GOVERNMENT PRINTING OFFICE.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., July 12, 1909.

Sir: I have the honor to transmit herewith and to recommend for publication as a bulletin of this Bureau a manuscript entitled "Cultural Studies of Species of *Penicillium*," by Dr. Charles Thom, mycologist in the cooperative soft-cheese investigations carried on at Storrs, Conn., by the Storrs Agricultural Experiment Station and the Dairy Division of this Bureau. A previous paper of this author dealing with certain species of *Penicillium* which are characteristic factors in the ripening of Camembert and Roquefort cheese has been published as Bulletin 82 of this Bureau. The investigations connected with the study of the organisms referred to necessitated the culture and comparison of many other species and an examination of the nomenclature for the whole genus *Penicillium*. Considerable confusion was found to exist regarding the identification of the various species, hence it seemed important that a comprehensive study from cultural data of all obtainable species should be undertaken.

Acknowledgment is made of suggestions and advice by Dr. Erwin F. Smith, of the Bureau of Plant Industry of this Department, besides the persons whose names are given in the text.

The illustrations, with one exception, are from original drawings by the author.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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CULTURAL STUDIES OF SPECIES OF PENICILLIUM.

INTRODUCTION.

In a previous paper [25a] two species of *Penicillium* were shown to secrete the proteolytic enzyms which ripen certain varieties of cheese. The effort to identify these organisms necessitated the culture and comparison of numerous other species and a study of the literature of nomenclature for the whole of the genus *Penicillium*. The difficulties encountered in deciding whether to discuss these forms under old names or to describe them anew from cultural data led to the extension of this study beyond the forms occurring in a dairy investigation so as to include any obtainable species, and especially all species whose identification under published names could be established.

Many recent studies have linked particular chemical and physiological activities with the presence of particular species of fungi. When such data relate to known species—identifiable species—our knowledge of the metabolism of these forms has been greatly increased. When, as has often happened, the generic name alone is given in a group so diverse in its activities as *Penicillium*, such data only add to the confusion. Whether the observations apply to all of the species of the genus or to a single unnamed species is left unsettled. To give real utility to such work, some one must test the applicability of the data to each species under consideration. On account of their ease of cultivation and the notion that there was a single common green species legitimately named *P. glaucum*, species of *Penicillium* have formed the subjects of many such investigations. In very few of these cases are sufficient data regarding morphology given to warrant even a guess as to the species used. An examination of the present status of specific nomenclature in the genus will therefore furnish a sounder basis for further studies of their activities.

This paper represents cultural work which has continued more than four years and includes those species for which the data obtained seem abundantly to justify the characterization offered. Some of these forms have been cultivated for the whole period, others for a less time. No claim to monographic completeness can be made. In

[a] The figures refer to the list of literature at end of bulletin.

collecting these forms, many of them have been isolated in this laboratory from dairy products and from fruits, or collected in the field by the writer. One series of forms was purchased from Kral, of Prague. Several species have been contributed by or verified by those who described them—two by Dr. G. Bainier, two by Dr. C. Wehmer, one by Dr. G. Delacroix, and one by Dr. G. G. Hedgcock. Many correspondents have sent cultures for examination or study.

THE BASIS FOR SPECIFIC CHARACTERIZATION.

The available sources of identification of these species will be discussed first. By the kindness of Professor Thaxter the exsiccati of the genus in the herbarium of Harvard University were examined. Cultures of certain species were tried but no living spores were found. No specimen was found in such condition as to be used to identify material by comparison. Similar courtesy was extended for the examination of specimens in the herbarium of Kew Gardens and at the University of Berlin. In no case was material found by which cultural material could be identified or identification verified by comparison. The plant bodies are too evanescent to retain the criteria necessary for identification for any great length of time, as ordinarily preserved. In most cases after a few years of handling the specimens were found reduced to powder. Wehmer[33] has noted for certain species of this genus that the conidia do not retain their power to germinate beyond a very few years. Specimens as formerly prepared therefore become useless for the identification of species by the method of types. The method described by Hedgcock[9] for preserving specimens has thus far been applied only to one species of *Penicillium* and entirely too recently to test its permanent value. Aside, therefore, from certain species which will be discussed later, it has been impossible to determine material belonging to this genus from herbarium specimens.

There remain two methods of identification: (1) The identification of material or cultures by the authors of descriptions; (2) identification by critical study of the descriptions and illustrations published.

Regarding the first method, fortunately some of the more recent authors have either preserved their organisms in culture, as is done at the Ecole de Pharmacie in Paris with the cultures of Bainier, or placed them in one other of the distributing laboratories where such organisms are maintained in continuous culture. But the earlier authors are dead and have left only their published descriptions and figures as a means of determining what organisms they studied.

Turning to the critical comparison of material with published descriptions, we find many difficulties. These descriptions give in meager outline observations of mold masses showing penicillate conidial fructifications, as found in nature upon substrata more or less

accurately specified. No cultures were made. The original masses are assumed to be comprised each of a single species. Parasitism or selective saprophytism is assumed, but the substratum is rarely designated with sufficient care to make a duplication of the original culture possible. Hence the characters would include whole series of forms whose differences are marked. No account is taken of their omnivorous nature nor of the marked variations introduced in appearance by changed conditions. The method of types and the method of substrata as represented by the herbarium material and the published descriptions we have are, therefore, not sufficient for the identification of species of *Penicillium*.

Although we abandon the "method of substrata" as the sole basis of description, the force of natural selection as shown by the distribution of certain of these species in nature is a most valuable accessory. Some species occur so constantly upon particular substrata and under particular conditions as to simplify their identification greatly. Examples of this are *P. italicum* Wehmer and *P. digitatum* Saccardo, as they are found upon citrus fruits. Unfortunately very few species restrict themselves to particular substrata, so that except for some few species and a very few media, the occurrence of a *Penicillium* in any given situation is but slight evidence for its identification. The constant occurrence of species of *Penicillium* in the laboratory, in connection with foods, and in factory processes, such as cheese-ripening, all point to controlled culture as the proper source of diagnostic characters.

If we look to cultural study[a] for our conception of species we have two methods of procedure: (1) The exhaustive study of the limits of variability for each species; (2) the comparative study of numerous species under arbitrarily chosen conditions uniformly maintained.

The first is the best method known for gaining complete knowledge of single species, but it is too cumbrous for taxonomic purposes. Physiological and chemical studies have commonly been restricted to particular classes of reactions for single species or groups of species. These have contributed much to our knowledge of fungous variability, but too often give no hint, except the vague nomenclature used, upon which to judge which species were actually studied. In experimental cultures changes in the chemical nature of the medium or in the conditions, or both, have been found to produce great changes in the morphology of the fungi studied. With the exception of a few fundamental group or generic characters, nearly every

[a] Weidemann,[35] in a recent paper, has described several new species of this genus in their relation to culture media. In this he has followed the bacteriologists further than the writer has thought necessary by giving formulas for a considerable number of media and detailed notes as to reactions upon such media, instead of the more formal descriptions attempted here.

attribute used in specific description has been shown to be a reaction to environment, hence changeable with such environment (for some species at least). Exhaustive study of all the species would be endless.

The alternative is to select certain media and a particular set of conditions, then to cultivate all the organisms under investigation in a uniform manner and to base distinctions of species upon differences in the reactions obtained, and upon the differing characters of the several species, in this common environment.

To test the reliability of such data, species obtained in the dairy laboratories were first studied carefully upon the peptone-milk-sugar gelatin described by Conn[4] and upon potato agar as described in our previous bulletin (Thom,[25] p. 7). From these cultures transfers have been made to media of very different composition—Cohn's solution, Raulin's fluid, milk in various forms, synthetic fluids presenting different sources of carbon—always bringing cultures back to the original media. Many species differ so materially in gross characters when grown upon these different media that successive cultures, if not known to be pure transfers, might be supposed to be different species; but when returned to the original media and conditions these forms have immediately produced the characters and reactions first found, with a large degree of uniformity. Absolutely uniform reactions are not to be expected from living organisms, at any rate under our imperfect control of working conditions; but when such reactions are definitely recognizable as essentially the same, the result may be judged as satisfactory. It is even more confusing to find that two or more species may react very similarly upon a particular substratum. A transfer of these organisms to a medium of markedly different composition brings out the contrasting characters, however. The desirability of recording the widest possible distinctions in such descriptive work makes necessary the use of media differing in composition as much as practicable, in such comparative cultures.

It is worthy of note that the species *P. roqueforti* and *P. camemberti*, essential to the cheese industry, have been isolated repeatedly from cheeses of widely different origin. The Roquefort species has been obtained from laboratories not concerned with Roquefort cheese studies, from ensilage, and from other substances. The same characters have been found in cultures of these two species from these variable sources under conditions in which the possibility of close genetic connection between cultures is thus remote. There seems to be little possibility of question that these species at least are well-fixed conidia-bearing forms, not cultural varieties of other species. The question at issue was not whether or how variations could be produced, but whether a particular variation is constantly produced

by a species in a particular environment. The correlative question whether the characters of a given species of mold can be permanently changed by passing through a series of cultures upon different media is involved in the same investigation. It is asserted by some workers that the physiological reactions of molds (if not also the morphology) can be changed, and that such changes persist after the return of the species to the original environment. So far as this investigation has gone such a view is certainly not supported by the conduct of the species of *Penicillium* which have been studied. In those species most thoroughly studied both the physiological and morphological reactions have appeared to be very reliable. A summary of these observations follows:

1. The same species may differ greatly in morphology and physiological reactions when grown upon different media.

2. Two species closely similar, when grown parallel in one environment, may differ characteristically when transferred to a different medium or a different set of conditions.

3. With these species the repetition of culture under particular conditions produces fairly constant morphology and reactions.

Forms arising in this way have been designated as ecads by Clements[3] in a recent discussion of the "Aspects of the species question." This name is used preferably for forms whose origin is known either because produced by cultivation or under such carefully determined natural conditions as admit of full description. With species of this genus in which part at least of the morphological characters of every culture are definitely attributable to the conditions and to the chemical character of the substratum, practically all known forms would therefore be properly designated as such ecads. If described as ecads, however, each description must be limited strictly to the data obtainable upon a single medium, whereas by writing into the description the reference to media it has been found possible to include a much more complete set of characters than would readily be worked out from a single set of conditions.

These observations lead to the conclusion that the cultural description of species of molds demands the recognition of the points noted below:

1. The culture media and conditions must be described so fully as to make the repetition of the culture upon the same medium and under approximately the same conditions easily possible anywhere.

2. The habit, structure, and appearance of the colony must be given as it develops upon at least two standard media of decidedly different composition.

3. The physiological effects of the colony upon these media should be noted.

4. Full drawings or photographs should show habit as well as microscopic details of cells and cell relations.

5. Other morphological or physiological data obtainable should be given as accessory information. Very striking characters are often found under accidental or unique conditions which immediately differentiate particular species. Some of these characters come up in the ordinary course of cultural study, others are found under accidental conditions but could rarely occur in laboratory routine.

It is clear that such descriptions can only result from repeated culture of a species under constant observation. If the range of such culture has been wide, it will bring out the most striking characters and thus reduce the number of minute distinctions necessary.

NECESSITY FOR DESCRIBING CULTURE MEDIA AND TEMPERATURE CONDITIONS.

In a previous paper[27] the characters available from cultural studies of species of *Penicillium* were discussed. The series of observations have been greatly extended in the three years intervening. These characters may therefore be profitably reviewed, following the summary just given.

Culture medium.—The composition of the substratum is shown in this paper to affect the character of the colonies grown upon it to such a degree as to make the exact description of the medium essential. Examples of these effects may be cited to show how conspicuous these differences may be. In a medium free from certain sugars *P. duclauxi* produces upon the surface of the medium very short conidiophores with conidial fructifications, whereas when such a sugar is added numerous coremia are formed, which in well-nourished colonies often become 10 mm. in height. This species also produces a rich purple color in certain media, but not in others. Hedgcock has noted that *P. aureum* Corda (as distributed by him) produces colonies orange-red upon alkaline media, but lemon-yellow in acid media. *P. digitatum* Sacc. grows sparingly, if at all, in media offering nitrogen only as nitrates, whereas many other species grow equally well from nitrates and organic nitrogen. One species is included which produces feeble gray or brownish cultures when carbon is presented from gelatin, starch, or lactose, but becomes a clear green when cane sugar is added.

A culture medium must offer not only the proper chemical elements for fungous growth, but must offer these in assimilable form. Different species of *Penicillium* make quite different demands as to the form of food substances required, hence a description must specify the substratum accurately enough to enable the duplication of culture conditions. If, however, the proper substances in the proper form are offered, changed percentages in the concentrations of these

substances have been found to affect the growth of cultures more slowly. Most species grow normally in extremely dilute solutions, but continue to grow well or even richly until the solutions reach concentrations of considerable osmotic pressure.

Temperature.—The species studied are mostly saprophytes of wide distribution. They are therefore able to thrive within a considerable range of temperature. Comparatively few of the species tested grew normally at blood heat (37° C.). At this maximum many of them either failed to grow or were actually killed. Within the range of 12° to 30° C. the rapidity of development in most species, as indicated by the production of colored fruit, increased with the rise in temperature. The lower temperatures affect the rate of fruit formation without, as a rule, preventing such development. Cultures held at 10° to 20° C. reached a development fully equal to those held at higher temperature, only in longer periods.

HABIT, STRUCTURE, AND APPEARANCE OF COLONIES.

Many series of comparative cultures indicate that any colony of a particular species will reproduce the same characters whenever grown upon a particular medium under particular conditions. The differences between many species of *Penicillium* are so striking to the eye as to enable one familiar with them to identify the several species immediately. These same differences are, however, so difficult to define, and in many cases so transitory, as to render their expression in form to insure recognition very difficult. Recognition of species depends at best upon a series of observations of these characters throughout one, and usually more than one, generation upon two or more substrata. A discussion of these characters follows:

Fruiting period.—In comparing cultures of related forms it is found that the time necessary for development from the spore to the production of ripe conidia differs for the different species, but that periods in the different species bear a comparatively constant relation to each other when all are grown under the same conditions. Similarly the length of the growing and fruiting period varies in the several species. In some species the mycelium produces new conidiophores and masses of conidia among or overgrowing the old for a considerable length of time. In some a secondary growth of white or even colored hyphæ often overgrows the fruiting area. In others the chains of conidia once started continue to increase in length for several weeks, until the conidial fructification becomes a mass 1 mm. or even more in length. In still others but a single crop of conidia is developed and the mycelium apparently dies or is totally exhausted in a few days. In some species the center of the colony matures and dies while the margin continues to grow and produce new conidiophores for some time. The habit of the species in the production of

conidial fruit is usually a well-marked character and is often found very useful in the separation of forms found growing together.

Color.—Color and color changes are difficult to describe on account of the deficiencies in the standards of color, but they form the first character noticed. The variety of greens, blue greens, gray greens, yellow greens, and shades of brown in the genus *Penicillium* baffles one seeking descriptive terms. The shades of color peculiar to each species under oft-repeated conditions are easily recognized and are quite reliable. The alterations in the color of spores due to changing the composition of the medium, as shown in the recent work of Stoll[24] and in this paper for species of this genus, and by others (Milburn,[15] Bessey[1]) for other genera, emphasize again the necessity of uniformity in and careful description of the culture medium. But in spite of the admitted difficulties in color description, the careful observation and record of the color of the colony at every stage of its development is very necessary to identification. This is complicated by the changes which occur at different ages of the colonies, so that it would be easily possible to place certain species in at least two of the color groups as designated by the older authors if we simply make our observations a few days apart.

The color of the mass of mycelium as observed from below ("reverse" as designated by Dierckx[5]) in cultures gives striking contrasts. This must not be confused with discoloration of the substratum which may or may not be produced by the same species in a given medium. The mycelium itself viewed in reverse has characteristic colors in certain species which are useful in diagnosis and are entirely independent of discolorations of the substratum. A colony colorless itself may color the medium brightly, while a colony bright colored itself may make no change in the color of the medium.

Surface.—As a convenient term "surface" may be used to designate the general appearance or the texture of the aerial portion of the colony. Perhaps the word "habit" would be in some measure more accurate, but that would apply also to the submerged mycelium. Comparison of the surface appearances of many cultures shows that this is one of the most stable characters when the same medium and conditions are used. For species of *Penicillium* and allied genera two types of surface will include most of the species met with.

In the one type all or a large majority of the conidiophores in the rapidly growing colony arise directly from strictly vegetative hyphæ which may be submerged in the substratum or lie upon its surface or be alternately prostrate and submerged. Each conidiophore stands separately, therefore, and usually all are found to be so nearly of the same length that the surface appears to be velvety. Such a surface may be called either velvety or "strict." A strict surface may be called "closely strict" when the conidiophores are so short

that the conidial fructifications are barely raised above the surface, or it may be loose or lax if longer conidiophores give a deeper velvety appearance.

In the other type all or most of the conidiophores are lateral branches of definitely aerial hyphæ. These hyphæ and conidiophores form felted masses or loose networks of aerial mycelium for which the term "floccose" is descriptive.

Margin.—In seeking the origin of all structures direct observation of the margin of the young and growing colony is essential. Such observation determines how the fungus spreads in the substratum, the septation and measurement of hyphæ, and the origin, order of development, and relative positions of aerial structures. As the colony matures growth ceases, ripe conidial areas extend to the very margin, the masses of conidia often change color and fall apart, and the conidia-bearing branches may curl up or drop off. Some species seem to inhibit their own further growth after a short period, while in other cases they dry up the culture medium by transpiring water. Many things thus contribute to render the old colony an unintelligible mass of spores and hyphæ.

In colonies with surface strict or velvety (consisting of conidiophores only) there is a succession of structures from the center to the periphery. In the center are conidiophores with ripe conidia, marked by the colored area. This shades into a white margin of developing conidiophores and conidial fructifications, while the extreme margin consists of submerged vegetative hyphæ. The relative width of these areas and rate of the development of conidiophores and colored conidia give characteristic appearances to colonies of particular species. In some there is a broad submerged vegetative border, then a similar white band of developing conidiophores. In others the area of colored conidia extends so closely to the margin that the white border is barely discernible. In the floccose species aerial mycelium often extends as rapidly at the margin as does the submerged part. In such cases the area of coloration follows the expansion of the colony more slowly.

Although close resemblances in culture are not uncommon, the relative development of these areas is quite typical and often sharply distinctive of species. Colonies showing a broad submerged and white margin usually spread over wide areas of the substratum, whereas those bearing ripe fruit to the very edge of the growing colony rarely develop beyond restricted areas.

The gross characters already discussed have purely specific value, or may even be more closely restricted as characters distinguishing particular ecads of species. The generic characters and very important specific characters are microscopic, and include cell relations and details of spore formation.

Conidiophore. The essential data as to conidiophores are their length, septation, the diameter of their cells, and especially their origin and relation to the substratum and to each other. Although extremes of variation in length of conidiophore may be very marked in any culture, the majority of conidiophores in any such culture approximate an average length. This length to be most reliable must be taken from the origin in another hypha to the lowest branch of the fructification. If the conidial fructification were counted into the length, the length of conidiophore would in many cases be doubled with the maturing of the spores. The actual length, however, is little changed with such maturity. Valid data on these points can be secured in many species only by direct observation of the undisturbed colony in the air under the microscope, instead of by the study of fluid mounts. This is equivalent to saying that Petri dishes, or other vessels which can be uncovered for study, must be used. The student must expect, therefore, to make many cultures and jeopardize the purity of one such culture every time he undertakes its proper examination.

Conidial fructification.—For lack of a better term conidial fructification may be defined as including the chains of conidia, the conidiiferous cells, and the branches bearing them back to their junction with the conidiophore proper. In a species of *Penicillium* fructifications vary greatly in detail, so that satisfactory illustration becomes difficult. Comparison of large numbers of fruiting branches in many cultures of the several species establishes specific types of appearance which can be shown in a conventionalized series of sketches for each species. The data found of value have been the mode of branching, the measurements of branches and conidiiferous cells, the relation of these to each other, the arrangement of the chains of conidia with reference to each other, and the measurement and appearance of the fructification as a whole. These penicillate fructifications vary from close columns of spores arising from single verticils of cells borne directly upon the apices of the conidiophores to widely spreading "brooms" whose numerous divergent chains arise from verticillately or complexly arranged branches from the original conidiophores. In the study of these conidial fructifications mounting in fluid commonly greatly disarranges these complex masses of branches and spore chains. Direct observation of the undisturbed colony is therefore essential to a correct conception of the habits of the species, though the details of branching and spore bearing must be sought in fluid mounts.

The term "conidiiferous cell" is used for the cell at the base of every chain of conidia in preference to "sterigma," as often used, or to "basidium" as used by Stoll.[24] The term was proposed by Professor Atkinson as meeting the objection that basidium implies a relationship not justified in fact, while sterigma usually designates not a spore-

producing cell but a spore-bearing process of a cell, hence is properly applicable to the narrowed apices of these very cells. In the Latin descriptions the term "basidium" has been retained, however.

Conidia.—The usual data with reference to the conidia are of equal service in our cultural studies, viz, shape, size, color, arrangement, markings, mode of germination, and conditions of growth. A record of the changes in color at different ages of the colony is essential. Completeness in observation is as necessary in spore characters as elsewhere. The variations in the size of conidia are notable in some species; in others conidia appear to be either globose or elliptical, even in the same chain. Where the outer cell wall is marked or spiny these markings often do not appear until the conidia are fully mature.

PHYSIOLOGICAL EFFECTS UPON MEDIA.

Certain physiological effects of fungous growth which are incident to the use of ordinary culture media have been found to be reliably characteristic of species. Some of these reactions are so conspicuous as to aid greatly in separating nearly related organisms. Such reactions as have been found uniform and definite or unique are introduced into the technical diagnoses of species in this paper. Other physiological data are appended as accessory cultural information.

Among the data observed in repeated series of comparative culture are the following: Odor, litmus reaction of medium at different stages of growth, liquefaction of gelatin media, the production of coloring substances in the media, the changes produced in milk, and the production of drops of transpired fluid upon surface of colony. Among the accessory data, observations upon carbon assimilation, upon proteolytic reactions, and upon the production of enzymes have been made for certain species.

Odor.—The production of definite odors by colonies is confined to a small number of species, and even among these often to particular media. When definitely present it is a character immediately recognizable and in certain species diagnostic; in others it associates the organism at once with a particular group of species.

Litmus.—The use of an indicator in the medium gives in very many species a sharp reaction. The value of this reaction is more narrowly restricted, however, than is indicated in previous papers (Thom[25, 26, 27]). The introduction of sterilized litmus or azolitmin into complex media brings contradictory results when the composition of the medium is slightly altered. If, for example, a solution of pure gelatin in distilled water be used as a nutrient, the reactions are definitely alkaline, with very few exceptions, which are just as definitely acid. The gelatin solution itself is acid. The alkaline reaction indicates that the products of the digestion of gelatin in such cases are of alkaline nature. If, however, the gelatin solution be neutralized and

5 per cent cane sugar be added, an equally large majority of the same species cause an acid reaction. Some few remain alkaline. These species growing upon cane sugar produce acids in quantity both to neutralize the alkaline products of the decomposition of gelatin and to change the reaction of the mass. The litmus reaction has been found generally reliable in a medium composed of 15 per cent gelatin in distilled water, in lactose-peptone gelatin after Conn's[4] formula, and in potato agar during four years of cultural work where many successive lots of media have involved the use of materials from different sources. In complex media the decomposition products of organic nitrogenous constituents and those of carbohydrates tend either to neutralize each other or to intensify the reactions, but add greatly to the difficulties of analyzing the results. The litmus reaction therefore may be used with the simplest organic media, but is best applicable to cultures in synthetic media where analysis of the results is feasible.

Gelatin.—The liquefaction of gelatin media by an organism in culture shows its ability to produce a particular form of proteolysis. This reaction is so conspicuous and so adaptable to cultural use that it has been recorded in all cultural studies. Various investigators have pointed out the limitations of this reaction. In cultivation nearly all of the species of *Penicillium* have been found to grow well upon a 15 per cent solution of gelatin alone in distilled water. Comparison of the results in this medium with liquefaction of several forms of gelatin media experimented with showed essential agreement. Since the advantages all lie in the simplification of formulæ, the data as to liquefaction by these species have been compiled from series of parallel cultures of all the species upon 15 per cent gelatin in distilled water.

The value of this reaction is measurably vitiated by the fact that any species which can subsist upon gelatin alone must be capable of more or less proteolytic action upon it. The results of observation confirm this statement. The value of these observations therefore depends upon the indication of the comparative rate of activity of different species in inducing proteolysis, not upon the presence or entire absence of this action. Numerous tests, involving a range from 15° to 25° C., show that slight changes of temperature affect this reaction only to the extent to which they affect the rate of growth, but do not disturb the comparative value of the data obtained. Within this range of temperature the most active species produce liquefaction in 5 to 8 days, other vigorous liquefiers require 8 to 15 days, while many species producing no liquid or but traces of softening in 15 days produce a gradual liquefaction in the succeeding 2 to 4 weeks. In studying species of *Penicillium*, liquefaction of gelatin has been found to separate such species as produce this proteolysis within

the time needed for other observations upon these cultures—about 15 days. Where liquefaction occurs during the active growth of the colony it definitely indicates the secretion of ectoenzyms capable of this digestion. Long-deferred liquefaction may result from such secretion or from the liberation of endoenzyms by the disorganization of mycelium.

Color production.—Certain species cause marked changes in color in some of the substrata in which they grow. Such reactions are usually produced upon certain media and not on others. They are therefore selective reactions. For example, one species produces a bright yellow color in media containing milk sugar, such as milk or peptone-milk sugar-gelatin; but no color in plain potato agar. Some of these colors are soluble in alcohol to form brightly colored solutions. The chemical nature of these substances has not been studied, but the reactions themselves have been thoroughly tested for a few species. In these species color production uniformly follows the proper culture conditions.

Production of fluid upon surface of colony.—The presence upon the surface of the colony of large drops of transpiration water which may be colorless or brightly colored by excreted products is common. Although a transient character dependent to some measure upon the humidity of the air in the culture vessel for its prominence, there is much difference between species in this particular.

Technical descriptions of these fungi have always been based, theoretically, upon morphology only. In such descriptions the host or substratum has been more or less definitely indicated. Where the organisms are parasites or saprophytes closely restricted to particular substrata and conditions, this practice can perhaps be justly supported. With omnivorous cosmopolitan saprophytes cultural study quickly shows morphology to be affected greatly by changed conditions or altered composition of media. Further, very many of these organisms have no known special habitat. A description based upon a specimen of one of these species found in the field, without comparative culture, might possibly contain some peculiar character which would identify a specimen of the same species when next found, but this is only a possibility. In practice the descriptions we have are nearly useless.

The introduction of cultural study carries with it the necessity of recognizing at least the most striking physiological data. With these species of *Penicillium* such data have been found as reliable as morphology. Further, the careful definition of morphology and reactions upon specified substrata under known conditions is only amplifying and rendering definite the two items—habitat and locality—which have always been included in plant descriptions. In recent years the students of ecology and of experimental evolution

have shown these to be much more important in flowering plants than was formerly supposed. The introduction of these characters is thus in full conformity with the best systematic practices of to-day, where the life history of the species is investigated as fully as possible.

CULTURE MEDIA.

In developing the descriptions presented the following media have been used:

1. Potato agar. This was made as described in another paper[25] (p. 7). Essentially this is simply a potato infusion to which agar-agar is added in any proportion desired. Since this medium is free from sugar and very dilute in nutrient value, sugar may be added to cultivate certain species.

2. Bean agar. The directions for making bean decoction were obtained from Mazé at the Pasteur Institute in Paris. Common white beans are heated in five volumes of water. Boiling is stopped just before the swelling of the cotyledons would rupture the seed coats. This gives a clear, slightly yellowish liquid which filters readily yet contains sufficient nutrients to grow many species normally. Agar may be added as desired. Since this decoction is poor in available carbon, the addition of sugar is often desirable for many species.

3. Peptone-milk sugar-gelatin, as described by Conn.[4] This standard bacteriological formula, with and without litmus, was used in developing the original draft of these descriptions.

4. Fifteen per cent gelatin in distilled water. As a stock medium, this was used without neutralization. Comparative cultures neutralized with NaOH seemed less adapted for most species than unchanged gelatin. With few exceptions the common species of *Penicillium* grow readily in plain gelatin and give the same reaction as in Conn's more complex formula.

5. Synthetic media. A. W. Dox, chemist to this investigation, has modified Czapek's formula for a synthetic medium, intended to present in a nearly neutral solution unaffected by sterilization the elements necessary for fungous growth. In stock form neither nitrogen nor carbon is presented in this fluid. It has consequently been found an excellent means of testing the availability of these elements in various forms. For convenience this will be referred to as Dox's solution, the formula of which is—

Distilled water	centimeters	3,000.0
Magnesium sulphate	grams	1.5
Dipotassium phosphate (K_2HPO_4)	do	3.0
Potassium chlorid	do	1.5
Ferrous sulphate	do	.03

In the work here reported nitrogen was added as sodium nitrate, 6 grams. Parallel experiments in which monopotassium phosphate (KH_2PO_4), which gives a perfectly clear solution of a strongly acid reaction, was substituted for dipotassium phosphate, K_2HPO_4, gave no advantages in culture to offset the advantages of a neutral medium except the disappearance of the traces of precipitated magnesium phosphate. The availability of carbon in any organic form can be tested readily in this solution. Solidified media are obtained by the addition of agar.

NOMENCLATURE.

THE GENERIC NAME.

The generic name, *Penicillium* Link, is held in this paper in its hyphomycete sense to designate all species which continue to propagate themselves for an indefinite number of generations by penicillate asexual fructifications. Such grouping does not imply the author's belief in the phylogenetic relationship of all such forms. The penicillate type of fructification is a definite character which binds together in this way into a "form-genus" a large number of cosmopolitan and omnivorous saprophytes, very few of which are known to produce sexual fructifications. Within this heterogeneous group, several series of forms possessing particular groups of characters have been separated and generic names have been based by some workers upon such segregation. Wehmer[28] founded the genus *Citromyces* upon two such species causing citric acid fermentation in sugar media, with its morphological basis in the presence of a single whorl of conidia-bearing cells at the apex of the conidiophore. Further study shows that the presence of this character alone would group together forms not so closely related to each other as to other species of *Penicillium* lacking this character. It seems best, therefore, to use the name *Penicillium* to designate the entire group and leave further investigation to establish permanent genera when really genetic relationships shall have been discovered.

NOMENCLATURE OF SPECIES.

In considering the actual problem of nomenclature of species several positions may be taken. It is not difficult to find published descriptions of single species in this genus which are sufficiently indefinite to include a large percentage of all the known species. As a rule, the morphological characters of the various forms under cultivation would not exclude them from half a dozen of the older species so far as current descriptions go. In many cases there would not, however, be the least reason for the adoption of one name in preference to another. Shall the investigator adopt for his material

a name previously used when he has little or no reason to believe that he has the organism originally described under that name? Granting the apparent impossibility of contradiction, he might find it a safe practice. If, however, contradiction should arise, the position becomes entirely indefensible. In case there is a fair probability that the forms are identical, the use of the old name may be justified perhaps without direct proofs. The alternative position calls for the complete description of the form studied and its distribution in culture to different centers of cultural work under a new specific name associated with this definite material and description. It can not be contended that these organisms are new to science, for it is entirely possible that certain of them have formed the basis for already published descriptions. This course can be justified by the contention that such publication applies to definite material, available for examination, culture, and comparison by others, and that there is less probability of confusion from such publication than would ensue from the use of names long published, without any real evidence of the identity of the organisms with those originally studied. After careful examination of all material available and consultation with many workers in closely similar fields, new names are attached to such forms as by continued cultural study appear to be sharply marked species but not identifiable by older descriptions.

The name *P. glaucum* is not used. Careful examination of literature and of cultural material from several sources, together with conferences and correspondence with investigators in numerous laboratories, does not afford evidence as to what form was originally used by Link or even secondarily by Brefeld[2] under this name. The name as used at present seems to be applied collectively to the common green forms which under examination are quickly found to be not one but several species. Further study may give some indication as to where the name really belongs, but until that time there is little profit in applying it to any particular form. It might, perhaps, be withheld until some worker succeeds in repeating Brefeld's classic studies in ascus production, and then applied to the form so found.

The present paper is not intended to be a monograph of the genus. It is presented as a report covering several thousands of cultures of a group of common forms, in the hope that the descriptions and key offered may be useful to others. The author has included studies of as many authenticated cultures of the more recently described forms as it was possible to secure. The verified cultures secured have already been listed. Much assistance and advice were freely given by Dr. C. Wehmer. On the other hand, it was impossible to secure cultures of the species recently described by Oudemans,[17] or those listed by Dierckx;[5] and one recently described by Peck[18] also appears to be lost. No claim to completeness can be

made, though the material may possibly form the nucleus of a real monograph later.

In the examination of the early literature of this genus the author is greatly indebted to Dr. C. L. Shear, of the Bureau of Plant Industry of this Department, for the use of books and for much careful cooperation in examining and discussing the subject of types and descriptions.

THE TYPE SPECIES.

Link[21] in his "Observationes," published in 1809, established the genus *Penicillium* to include the common green molds having a penicillate type of conidial fructification—a conidiophore branching more or less complexly at its apex, such branches becoming or being tipped by cells, each of which produces a chain of conidia. The whole produces a brush-like appearance, the chains of conidia serving as the hairs or bristles of the brush. This generic name has been universally accepted to include in a form genus all species reproducing themselves indefinitely by such conidial fructifications. However doubtful we may be as to the forms originally examined, there is no question that we know the general type of structure which Link intended in his description of the genus.

Under the genus *Penicillium*, Link placed three species. The first species listed was *P. glaucum*, but the description given is equally applicable to many different forms. This was noted as frequent in decaying bodies and said to be most closely related to *P. expansum* (the third species listed), of which he suggests his material may have been but undeveloped specimens.

The second species, *P. candidum*, is described as producing round colonies, with mycelium and spores white, upon decaying fungi and herbs. Although many authors have used this name for material from different sources, and Morini[16] has described an ascigerous form under this name, there has been no means of determining what form Link had in mind when writing his description. Many forms will produce white mycelium and spores under special conditions, while but one of those examined has been shown to do this under all conditions, and this one is so specialized in its habit as to be excluded by Link's statement of habitat from the application of this name. Numerous authors have suggested that the *P. "candidum"* forms are probably colonies of species, colorless under special conditions, but green under other cultural conditions.

The third species, *P. expansum*, is slightly better described, while its habitat is primarily given as rotten fruit, although the author has manifestly extended his use of the name to forms growing upon other substrata which he believed to be identical with the fungi grown upon decaying fruit.

On page 19 of his "Observationes" Link describes the genus *Coremium* with a single species, *C. glaucum*, which he specifies as found upon decaying fruits. This fungus can be traced through a connected series of publications giving descriptions and figures which show the original conception to include (if not entirely to be drawn from) the large green coremia which develop upon apples and related fruits decaying in storage.

This organism is figured by Greville[8] as *Floccaria glauca;* it is cited cited by Fries[7] as a variety of *Penicillium crustaceum*, from which he has "seen it originate upon apples in the autumn." It is twice referred to by Corda in Icones Fungorum (Vol. II, p. 17); he cites *Coremium glaucum*, *C. citrimum*, and *C. candidum* as synonyms; again in Prachtflora (p. 54, taf. XXV, figs. 3, 4, 17, 18, 19, 20, and 21) under the name of *Coremium vulgare* he manifestly had this same species, although he groups it with figures which appear to be different organisms.

These figures and descriptions cited are definite enough to show that workers contemporary with Link applied the name *Coremium glaucum* Link to the coremiform rot of the apple. As a result of observations of living material Fries considered this only a form of *Penicillium crustaceum*, which he made to include *P. glaucum* and *P. expansum* Link.

The present writer has collected this fungus upon decaying apples and related fruits repeatedly in America; also upon pears and mespilus in Hanover, Germany.

Repeated cultures have shown that the ability to produce coremia is a definite character of this species, recognizable under many conditions of culture, but not shown under other conditions. The same culture will commonly show both simple penicillate fructifications and coremium production. The species must therefore be regarded as one of the several species of *Penicillium* which always produce coremia under proper cultural conditions.

Examining Link's species of *Penicillium*, we find that he specifies *P. expansum* as primarily found upon rotten fruit. *P. expansum* Link clearly included *Coremium glaucum* Link, therefore, probably with others; but from its known abundance in Germany there can be little question as to this organism forming in part, at least, Link's original conception of this species. Later (1824) in Species Plantarum, Tomus VI, page 70, Link[11] redescribes *P. glaucum* and includes in it the *P. expansum* of his Observationes. In this discussion Link broadens his description of *P. glaucum* to include all green forms found in decaying substances, upon the assumption that all such forms are but a single species. It is evident that the earlier description *P. expansum* Link included this species with sufficient restriction

to justify reviving the name *P. expansum* Link and limiting it to the penicillium rot of apples alone.

P. expansum Link (in part), the penicillium rot of apples, would therefore stand as the type species of the genus.

The technical rules for the establishment of type species are in this way satisfied. The kind of plant used by Link is perfectly well known. To say just which forms he had in hand may be impossible, but the form we are discussing was certainly one of them.

EXPLANATION OF DRAWINGS.

The drawings of the species described have been made with the Bausch & Lomb camera lucida in all cases except such as are marked diagrammatic or partly diagrammatic.

For those with the magnification of 140, the lenses used were the Bausch & Lomb 1-inch ocular and the two-thirds objective; for those of 900 the lenses were the Bausch & Lomb 1-inch ocular and the one-eighth objective; for those of 1,400 the lenses were the Bausch & Lomb 1-inch ocular and the one-twelfth objective; for those of 1,600 the Spencer No. 12 compensating ocular and the Zeiss 3 mm. apert. 1.30 apochromatic. This apochromatic was also used in a few cases at a magnification of 900; these are indicated in the legends. All figures at magnification of 140 were drawn from the exposed surface of the undisturbed colony in Petri-dish cultures. All other figures were made from fluid mounts or hanging-drop cultures (for spore germinations).

The series of sketches at 140 magnification are made with uniform methods, so that comparison of species in culture can be much easier than from figures made at different magnifications.

PENICILLIUM EXPANSUM Link.

P. expansum Link (in part), emended Thom=penicillium rot of apples and allied fruits.

Syn. *Coremium glaucum* Link, Observations, p, 19; Icones, V, fig. 31, 1809.
Floccaria glauca Greville, Scottish Flora, pl. 301, figs. 1–4.
Penicillium glaucum Link (in part), Species Plantarum, VI (1824), p. 70.
Coremium vulgare Corda (in part), Prachtflora, p. 54, Pl. XXV, especially figs. 3, 4, 17, 18, 19, 20, and 21.
Possibly *P. elongatum* Dierckx.

Colonies upon gelatin and potato or bean agar, green becoming gray-green and slowly brown in several weeks (especially when exposed to light), floccose, with concentric zones tufted with short, loose, coremium-like aggregations of conidiophores, not over 1–2 mm. in height except in old cultures containing sugar, broadly spreading with broad white margin in growing colonies. Reverse somewhat brown. Conidiophores either very short lateral branches of aerial hyphæ or very long (1 mm. or more), arising singly or grouped with others to form coremia. Conidial fructifications consist of 1 to 3 main branches bearing verticils of branchlets supporting crowded whorls of conidiiferous cells, 130–200 by 50–60μ at base in cultures without sugar, with sugar

continuing for some weeks to produce great numbers of conidia which come to form masses perhaps 1 mm. in thickness. Conidiiferous cells 8-10 by 2-3μ. Conidia elliptical to globose 2 by 3.3μ or 3-3.4μ; green, homogeneous, persisting in chains when mounted. Colonies begin to liquefy gelatin slowly after about 10 days and continue until it is completely liquefied. Grows readily and rapidly upon all common media.

Occurs characteristically upon decaying apples and other pomaceous fruits, where old colonies often produce coremia 1 cm. or more in length and very large.

Collected at Ithaca and Geneva (Eustace), N. Y., at Middletown and Storrs, Conn., upon apples; upon pears and Mespilus at Hanover, Germany. Often appears as a contamination in fungous cultures.

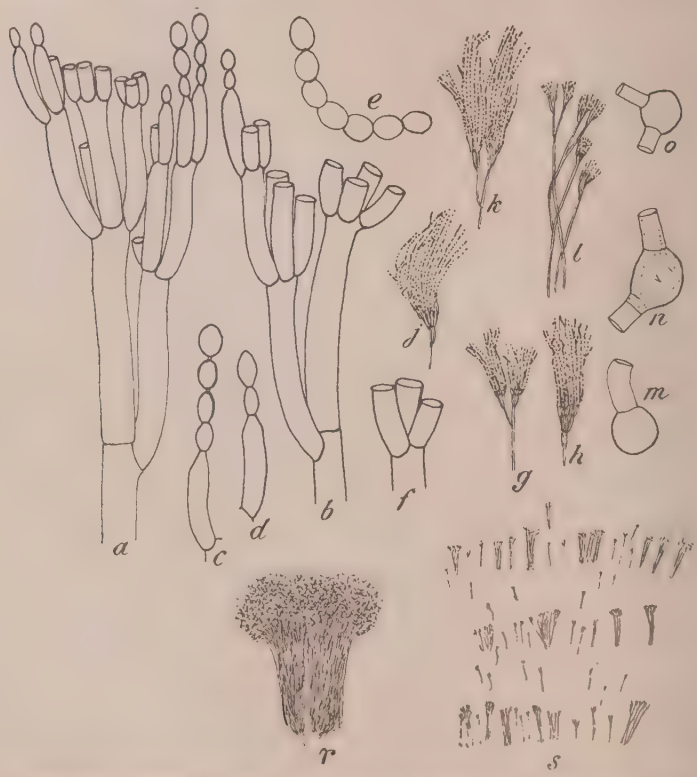

FIG. 1.—*Penicillium expansum* Link: *a*, *b*, *f*, branching and arrangement of branches of conidial fructification (× 900); *c*, *d*, *e*, conidiferous cells and conidial chains (× 900); *g*, *h*, *j*, *k*, *l*, sketches of fructification (× 140); *m*, *n*, *o*, germination of conidia (× 900); *r*, *s*, sketches from photomicrographs, showing in *s* loose aggregations of conidiophores beginning to develop in zones which become coremia like *r* (coremium *r* was 1 mm. in height; × 35).

Many references in the literature to *P. glaucum* Link and *P. crustaceum* (L.) Fries refer to this species. Unless, however, such citations directly refer to the presence of coremia, or to the association of the organism with the decay of pomaceous fruits, or both, there is no means of fixing the application of such names to this species. Since Link[11] in 1824 lumped into his species *P. glaucum* every kind of green

penicillium for which he could find references, the myth of "the common green mold" seems to have had pretty general acceptance, although we find here and there a protest against this view.

A culture of this species can always be obtained from apples decaying in storage, upon which usually well-developed coremia can be found if proper search is made. The wide distribution of the organism as noted above and as seen in the literature justifies belief in its general distribution. Once carefully observed in cultures, the investigator will usually recognize the organism on sight when it appears in his cultures even as a contamination of other species. The odor of this species is so distinctive as to assist greatly in identifying it in accidental cultures. It does not produce the yellow color in the substratum as described by Lindau[10] for *P. glaucum*, nor are its spores globose from the first as Wehmer[30] records them for that species. It seems so very well characterized as to justify sharply separating it from the other green forms.

CULTURAL DATA.

Color, green or gray-green; color of reverse, yellowish to somewhat brown; color in media, colorless or yellowish brown (milk).

Odor, "fruity" in all or nearly all cultures.

Fifteen per cent gelatin in water, good growth, with loose and ill-defined but abundant coremia; liquefaction slow, more or less liquid in 2 weeks; litmus reaction alkaline or neutral. Potato agar and bean agar, characteristic colonies, gray-green with more or less coremiform bundles of conidiophores. Potato plugs, characteristic colony.

Raulin's fluid agar, characteristic colony with concentric rings of broad coremia. Raulin's fluid, characteristic colony. Cohn's solution, slight growth, few coremia, brownish below.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, good coloring up to 50 per cent sugar, acid reaction, conidiophores in dense areas with few definite coremia. Lactose 3 per cent, slow development, about half normal. Levulose 3 per cent, typical, alkaline, or neutral reaction. Galactose 3 per cent, typical, acid reaction. Glycerin 3 per cent, slow development, increased greatly by adding sugar. Potato starch 3 per cent, fair growth, no coremia. Butterfat, rich typical growth.

Milk, typical colonies, coremia in a ring at glass; curdling (0.25 per cent calcium chlorid added) in 1 week; digestion rather slow; color in milk yellowish brown.

At 37° C., no growth, culture grew when cooled; at 20° C., good growth.

The coremia of this species are especially characteristic of old cultures in which drying has begun. In fluid cultures they are commonly attached to the glass above or at the very top of the fluid.

PENICILLIUM ITALICUM Wehmer.

Beitr. z. Kennt. einh. Pilze, Jena, 1895, p. 68, t. II.

Colonies on plain gelatin and potato or bean agar bluish green, becoming gray-green when old (bright bluish green on cane-sugar media and citrus fruits), broadly spreading, aerial portion composed at the broad margin almost entirely of conidiophores, but becoming slightly floccose in the center. Reverse of colonies dark brownish, often almost black in media containing sugar. Conidiophores from short (100μ) to very long (600μ), averaging perhaps 250μ, arising either directly from substratum or as branches of aerial hyphæ. Conidial fructifications up to 300μ or more in length, consisting usually

of a main branch and one lateral branch, each producing a whorl of branchlets bearing crowded verticils of conidiiferous cells, 12–14 by 3μ. Conidia breaking off in masses in handling old cultures, which rise in clouds when shaken. Pronounced odor in cultures containing cane sugar. Chains of conidia loosely divergent, long; conidia 2–3 by 3–5μ, cylindrical to elliptical or slightly ovate, clear green by transmitted light, very variable in size but usually within the limits given. Masses of spores continue to increase from 2 to 3 weeks. Petri-dish colonies partially and slowly liquefy gelatin (12 to 20 days). Numerous white sclerotia are produced upon the surface of

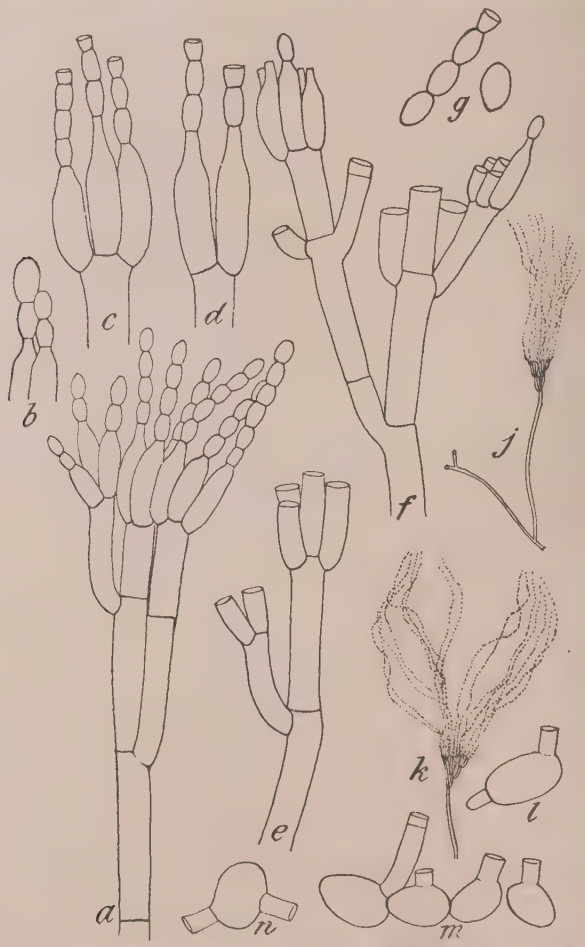

FIG. 2.—*Penicillium italicum* Wehmer: *a, b, c, d, e, f, g*, types of branching, formation of verticils of conidiiferous cells and conidial chains (*a, e, f*, × 900; *b, c, d, g*, × 1,400); *j, k*, sketches of conidial fructifications (× 140); *l, m, n*, swelling and germination of conidia (× 900).

the medium after 2 to 3 weeks' growth, especially upon fruits and other acid media rich in sugar.

Cosmopolitan; characteristic of decaying oranges, which become bright blue-green with this mold as contrasted with the olive-green species which is often associated with it upon the same fruit. These contrasting colors are illustrated by Wehmer.[31]

In his discussion of this fungus Wehmer[31] shows that the blue-green rot of citrus fruits is a different species from the similarly colored apple rot. His description seems to be the first recognition of this species as different from the green molds occurring constantly upon all kinds of food. This species has been discussed as *P. glaucum* in recent papers (R. E. Smith,[22] Powell[20]), where its agency in the decay of citrus fruits is very fully considered.

Cultures were obtained by the writer from oranges in Hanover, Germany, and identified by the describer, Dr. C. Wehmer. It has since been repeatedly observed and collected in America. Pure cultures can always be secured by finding decaying oranges in the market which have the blue-green areas of rot just beginning to appear upon them. These areas are usually blue-green in center surrounded by white areas which are usually grouped into little white patches toward the vegetative margin and the whole superficial colony surrounded by an area of soft, watery rot. Very often such colonies when older become much contaminated with the olive-colored rot, given in this paper as *P. digitatum*.

CULTURAL DATA.

Color, clear bluish-green on sugar media, shades of gray-green without sugar; reverse of colony commonly brownish in areas; color in media none or slight.

Odor distinct upon media containing cane sugar, none on lactose or media free from sugar.

Fifteen per cent gelatin in water, medium growth; liquefaction, none until several weeks old, then partial in acidified cultures; litmus reaction fairly alkaline. Potato agar with lactose, rather thin gray-green colonies, not vigorous, acid reaction. Potato plugs, pale but characteristic. Raulin's fluid, typical colonies. Cohn's solution, germination only.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, good colonies up to 50 per cent with alkaline reaction. Lactose 3 per cent, slow growth but typical, with acid reaction. Lactic acid 0.9 per cent, small growth. Levulose 3 per cent, not normal. Galactose 3 per cent, medium growth, acid reaction. Glycerin 3 per cent, no growth; growth began when sugar was added. Butterfat, growth slow.

Milk, good growth; curdling (0.25 per cent calcium chlorid added), ten days; digestion partial and slow; color slowly brownish or yellow-brown.

At 37° C., killed; at 20° C., good growth.

PENICILLIUM DIGITATUM Saccardo.

The olive-green orange rot, *P. digitatum* Sacc., in Mycotheca Italica, No. 986, Herbarium U. S. Department of Agriculture; in Sylloge Fungorum, Vol. IV, p. 79; in Fungi Italici, No. 894.

=*P. olivaceum* Wehmer, Beitr. z. Kennt. einheim. Pilze, pp. 73, t. II, Jena, 1895.

?*Mucor cæspitosus* L., in Species Plantarum (1753), II, p. 1186, based upon Micheli, tab. 91, fig. 3.

?*Monilia digitata* Fries, Systema Mycologicum, III, p. 411.

Colonies on sugar gelatin and potato or bean agar grayish olive, irregularly shaped from the unequal growth and branching of rather few hyphæ, aerial portion consisting only of very short conidiophores and conidia. Reverse of colony commonly shows brown to black colors. Conidiophores rising directly from the substratum, 30–100 by

4–5μ, usually very short. Conidial fructification a few tangled conidial chains up to 160μ in length, borne upon conidiiferous cells 13–16 by 3–4μ. Conidia cylindrical to almost globose, 4–7 by 6–8μ (at times 6 by 10μ), often uneven in size and shape in the same chain. Colonies do not liquefy sugar gelatin except at times partially in cultures three weeks old or more. Litmus reaction acid. Grows readily on organic media, but shows a very pronounced affinity for such media with high percentages of sugar, in which it produces a strong odor. Refused to grow in synthetic media containing nitrogen as sodium nitrate.

Cosmopolitan upon citrus fruits, distinguished from *P. italicum* by the sharp contrast of its olive color with the blue of the other. Collected in Hanover and verified by Dr. C. Wehmer. Received from Prof. P. H. Rolfs in Florida. Seen upon decaying oranges everywhere. Pure cultures can always be secured from the common market fruits.

Nomenclature.—Wehmer [31] (1895) gives the first adequate discussion of the decay of citrus fruits by the agency of species of *Penicillium* in which the forms found were shown to be distinct species associated constantly with these fruits instead of common green species accidentally occurring upon these fruits. For the olive-green form the name proposed, *P. olivaceum*, is descriptive, but had already been used by Corda (Icones, III, p. 12, t. II, fig. 35) for a species afterwards transferred to *Hormodendrum*. The name *P. olivaceum* Wehmer is therefore not tenable by present rules of nomenclature.

P. digitatum is the name used by Saccardo, as is shown by the specimens distributed by him (Mycotheca Italica, No. 986) previous to Wehmer's work. In his description and synonymy he cites the name from Fries (Systema Mycologicum, p. 411), where it appears as *Monilia digitata*, transferred by Saccardo to *Penicillium*. But Fries cites his use of the name from Persoon [19] (Synopsis Fungorum, p. 693), who bases his description of *Monilia digitata* upon Micheli's [14] figure and description (Nova Plantarum Genera, p. 213, pl. 91, fig. 3). Here, under the name of *Aspergillus*, Micheli figures and describes as No. 8 a fungus which may be taken for a *Penicillium*, which is said to have been found upon semi-putrid lemons. This figure is cited by Linnæus in Species Plantarum (1753), II, p. 1656, as the basis of his *Mucor cæspitosus*.

If we accept Saccardo's citations as correctly giving the origin of the name and tracing it back to Micheli, we must abandon the name he uses and adopt the name given by Linnæus—*Mucor cæspitosus* = *P. cæspitosum* (L.). Careful scrutiny of Micheli's figure gives no possible means of identification. Aside from identification from preserved specimens which are not cited by the authors in any case, there is little possibility of showing what any of the authors commonly cited, for this species may have had until we come to the description given by Saccardo in the Sylloge (IV, p. 79), and the material distributed by him in Mycotheca Italica, which I have seen and which is certainly this fungus. I have therefore continued the use of Saccardo's name in this case because he has given the first

description based upon tangible material, but have rejected his citations. The following are the principal citations which have been given as referring to this species:

Micheli, Genera Plantarum (1729), pl. 91, fig. 3.
Linnæus, Species Plantarum (1753), II, p. 1656.
Persoon, Observations, p. 41.
Persoon, Synopsis Fungorum, p. 693, *Monilia digitata.*
Fries, Systema Mycologicum (1829), III, p. 411, *Monilia digitata.*
Saccardo, Fungi Italici (1881), No. 894, and Sylloge, IV, p. 79, *P. digitatum.*
Wehmer, beitr. z. Kennt. einheim. Pilze, p. 73, taf. II.

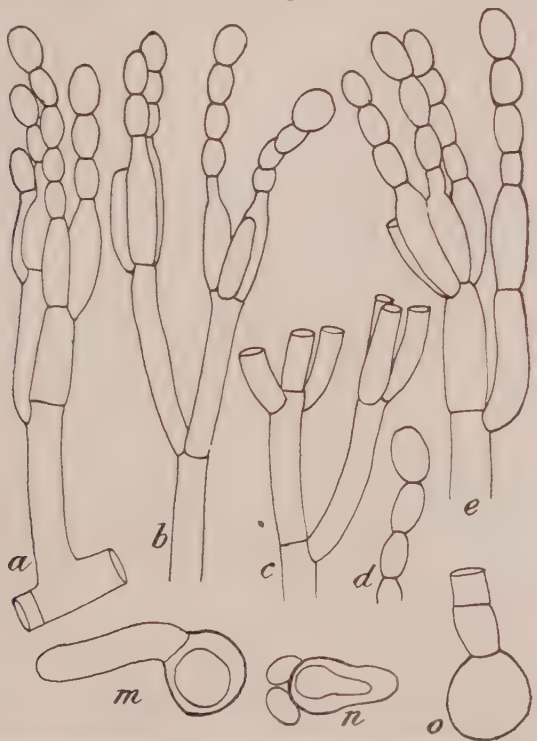

FIG. 3.—*Penicillium digitatum* Saccardo: *a*, whole conidiophore and fructification; *b*, *c*, *d*, *e*, types of branching and formation of conidia; *m*, *n*, *o*, germination of conidia; all × 900.

The only reason for retaining the name *P. digitatum* and ascribing its authorship to Saccardo is that the description cited is the first one which we can ascribe definitely to material of this species. If, as said above, his citation of literature proves to be correct, the name he gives becomes untenable by present rules of nomenclature, and must be changed to *P. cæspitosum* as given by Linnæus, although there is no evidence that Linnæus actually examined material of this species. Since there is more or less doubt about usages previous to Saccardo, and since the name given by Saccardo has become asso-

ciated with studies of this species in recent literature (Powell,[20] Smith [22]), this name is allowed to stand, at least until we have more information.

CULTURAL DATA.

Color, shades of olive green; reverse brown or dark brown, especially in sugar media; color in media, none or slightly yellowish.

Odor, associated with smell of rotting oranges, strongest upon cane-sugar media.

Fifteen per cent gelatin in water, weak growth, not adapted for this species; liquefaction none, slowly accomplished in gelatin to which sugar is added; litmus reaction acid. Potato agar and bean agar, colonies as described above, rather poor growth which becomes enormously increased upon the addition of cane sugar. Potato plugs, excellent growth. Raulin's fluid, colony colorless. Cohn's solution, germination only.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, germinated only. Lactose 3 per cent, germinated but no growth. Lactic acid, 0.9 per cent, germinated only. Levulose 3 per cent, germination only. Galactose 3 per cent, slight growth. Glycerin 3 per cent, germination. Potato starch 3 per cent, slight or no growth. Butterfat, germination only. Conidia inoculated into this fluid with various sources of carbon germinated but never developed normal colonies.

Milk.—This species never produced vigorous growth upon milk.

At 37° C., killed; check at 20° C. grew well.

PENICILLIUM ROQUEFORTI Thom.

U. S. Department of Agriculture, Bureau of Animal Industry, Bul. 82, pp. 35–36, fig. 2, 1906.

Report Storrs Agricultural Experiment Station, 1905, p. 111.

Syn. *P. glaucum*, various authors—not Link or Brefeld.

Colonies on potato agar or lactose gelatin quickly turning green, becoming a dirty brown when old, velvety strict, indeterminately spreading by large main radiating, branching hyphæ, giving a somewhat uneven or indefinite margin, which gets a white, fibrous, almost spider-web appearance from its alternation of submerged parts of hyphæ with short prostrate aerial loops. Reverse of colony yellowish white. Conidiophores arising separately and in acropetal succession from the growing parts of submerged hyphæ (comparatively few aerial parts, but some), 200–300μ septate. Fructification 90–120μ or at times 160 by 30–60μ at broadest place, usually appearing double by the divergence of the lowest branch; branchlets ("basidiophores") irregularly verticillate, bearing crowded verticils of appressed conidiiferous cells (basidia), 9–11 by 2.5μ with long, divergent chains of conidia. Conidia bluish green, cylindrical to globose, smooth, rather firm walled, 4–5μ in diameter, germinating by a straight tube. Colonies do not liquefy sugar-gelatin, though they soften it somewhat. Fungus on plain gelatin or potato agar changes litmus from red to blue very rapidly and strongly almost from the beginning of the growth. Fruiting period short, but one crop of spores upon the mycelium. Cosmopolitan and omnivorous, or nearly so. Characteristic of Roquefort and related types of cheese.

The mold of Roquefort and related types of cheese has been commonly designated in dairy literature as *P. glaucum* Link. Such citations refer to this fungus, though many times referring to it as "the common green mold." Although it is not restricted in its habitat to cheese, this species is so identified with the ripening process of Roquefort cheese (in which pure cultures are used) that any one

desiring a culture of this species can always obtain it by purchasing cheese of this type. It also occurs frequently in ensilage and is often found as a contamination in laboratory cultures of other fungi. The species is well marked by cultural characters from which it can be readily recognized when once studied. Its agency in the ripening of Roquefort, Gorgonzola, and Stilton cheeses is discussed in another paper (Thom[25]).

CULTURAL DATA.

Color, gray-green to clear green, becoming brownish when old if exposed; reverse colorless or cream; color in media, none, or in some cases a slight and evanescent greenish tinge.[a]

Odor, none (unless accountable for some odor in Roquefort cheese).

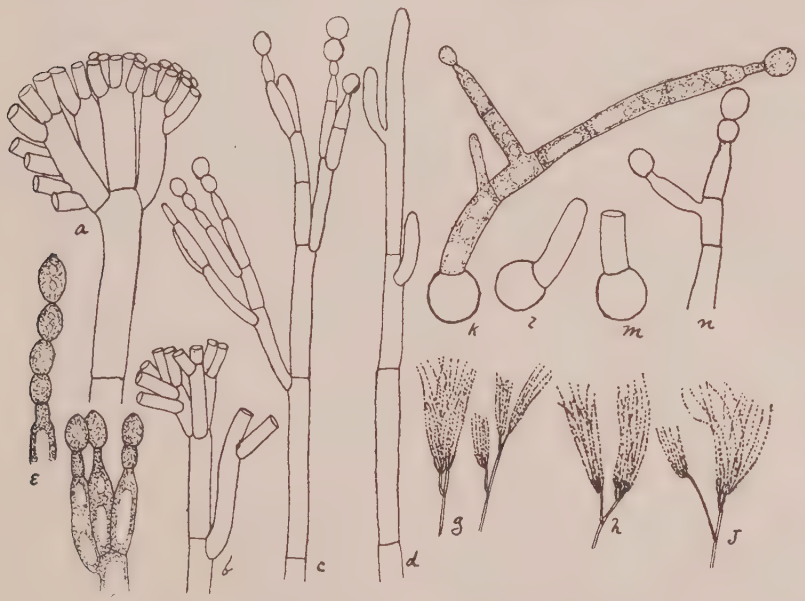

FIG. 4.—*Penicillium roqueforti* Thom: *a*, part of conidiophore and of base of fructification, highly magnified, showing production of basidia on sides as well as at apex of basidiophore; *b*, *c*, other types of branching; *d*, young conidiophore just branching; *e*, *f*, basidia and the formation of conidia, highly magnified; *g*, *h*, *j*, diagrams of types of fructification as seen under low power (× 80); *k*, *l*, *m*, *n*, germination of conidia and new conidia produced directly on the first hyphæ. (From Bulletin 82, Bureau of Animal Industry.)

Fifteen per cent gelatin in water, good growth, not heavy; liquefaction, none, or partial after 2 to 3 weeks in acidified cultures; litmus reaction, alkaline. Potato agar and bean agar, gray-green, loose, becoming dense and deep green when sugar is added. Potato plugs, characteristic. Raulin's fluid, very dense, deep green colonies. Cohn's solution, slight growth.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, slow growing but typical colonies up to 30 per cent, persistently green, not acid. Lactose 3 per cent, weak

[a] Weidemann[35] records the production of a red color in cane-sugar solution by this species. The writer has not obtained this color in his cultures.

growth, evidently carbon deficiency. Lactic acid 0.9 per cent, better than lactose, not typical colony, good color. Levulose 3 per cent, weak colonies. Galactose 3 per cent, good growth, alkaline. Glycerin 3 per cent, weak growth. Alcohol, good growth, becoming brown in age. Tartaric acid, very slight growth. Butterfat, rich growth.

Milk, growth good, alkaline to litmus; curdling (0.25 per cent of calcium chlorid added) in 10 days; digestion, fairly rapid; color in milk, none.

At 37° C., no growth; grew when cooled; check at 20° C., good colony.

PENICILLIUM PURPUROGENUM O. Stoll.

Beitr. z. morph. u. biol. Char. Penicillium, Würzburg, 1904, p. 32, t. I, fig. 6; t. III, fig. 2; t. IV, fig. 3.

Colonies on lactose gelatin and potato or bean agar, gray-green to brown or olive, deeper green upon cane-sugar media, closely floccose, almost velvety in surface appearance, spreading slowly over the substratum and producing in the whole mass of medium a red color. In acid media rich in sugar secondary floccose mycelium arises white or with hyphæ studded with yellow granules. Conidiophores 100–300 by 3.5μ, arising separately or from portions of hyphæ just above the surface of the substratum. Conidial fructification 50–100μ in length, composed of one verticil of branches (sometimes with a secondary or partial secondary verticil), bearing whorls of conidiiferous cells 11–12 by 2.5μ, narrowed abruptly to form sterigmata at the apices. Chains long, divergent. Conidia elliptical, 3.4–3.8μ by 2–2.5μ, green, granular, with from one to several small highly refractive granules in each, in chains falling apart in fluid mounts. Colonies liquefy sugar-gelatin slowly in 15 to 20 days.

Received from Kral in Prague.

The authority for the name of this species is attributed by Saccardo to Otto Stoll, as here indicated, since his description, or rather discussion, of this fungus forms the basis of Saccardo's Latin diagnosis. Stoll quotes the name from Kral, who gives the author as Alex. Fleroff, in Warsaw. Stoll has given the first discussion that is in any way adequate.

A closely similar organism has been found by Prof. F. D. Heald upon corn (*Zea mays*) in Nebraska. A third form corresponding closely in morphology and many cultural characters was sent from Miami, Fla., by Professor Rolfs. Although distinguishable by some characters, these forms resemble *P. purpurogenum* as described above so closely in morphology and cultural characters as to justify including them, temporarily at least, under this name. Neither of these forms produces the purple color as rapidly or as purely as the original race of *P. purpurogenum*.

CULTURAL DATA.

Color gray-green, becoming dark green with the presence of cane sugar; reverse yellow to reddish, or colorless; color in media, none to red to deep purple, almost black, according to medium; odor, none.

Fifteen per cent gelatin in water, medium growth, characteristic fruiting; liquefaction, partial in cultures 3 weeks old, or none, none in 10 to 12 days; litmus reaction, slowly alkaline or often neutral. Potato agar and bean agar, good colonies but no purple color, purple produced when sugar is added. Potato plugs, mycelium yellow

with granules, potato becoming purple. Raulin's fluid, slow development. Cohn's solution, germination only.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar 10 per cent in agar, rich growth, deep red medium. Lactose 3 per cent, very little growth, very pale purplish. Lactic acid 0.9 per cent, medium growth, slowly producing purple in medium. Levulose 3 per cent, small colonies, fluid pale red. Galactose 3 per cent, small colonies, acid reaction. Glycerin 3 per cent, no growth. Alcohol 3 to 5 per cent in agar, good growth but slow, medium becoming purple. Potato starch 3 per cent, good growth, abundant purple in medium. Tartaric acid, no growth. Butterfat, very slow growth with purple color in fluid.

Milk, slow development; curdling (0.25 per cent calcium chlorid added) 13 days; digestion slow and only partial; color in milk, shades of purple according to progress, deepest under colony.

Cooked apple, feeble growth.

Grew equally well at 37° C. and 20° C.

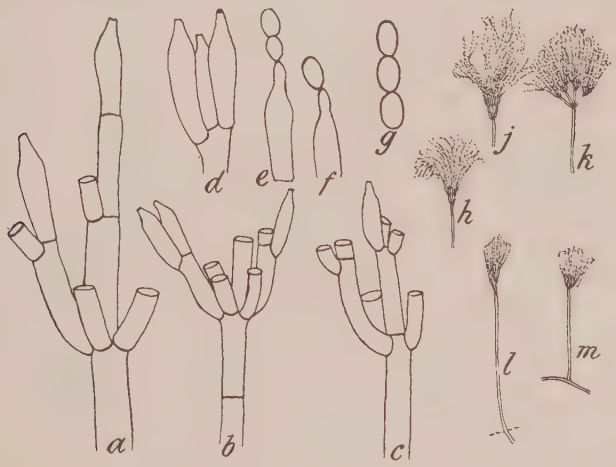

FIG. 5.—*Penicillium purpurogenum* O. Stoll: *a, b, c,* conidial fructification showing branching and arrangement (× 900); *a,* form with partial secondary verticil; *d, e, f, g,* conidiiferous cells and conidia (× 1,400); *h, j, k, l, m,* sketches of whole fructifications (× 140).

PENICILLIUM PINOPHILUM Hedgcock (nomen novum).

Syn. *Penicillium aureum* Corda, emended Hedgcock, Mo. Bot. Gard. Rept. 17, pp. 105–107, pl. ii, figs. 1–3.

Not *P. aureum* Corda, Prachtflora, p. 38, t. XVIII.

Colonies on potato or bean agar and milk sugar gelatin, from green on agar through shades of yellow-green to bright yellow and orange on media containing starch and cane sugar. Superficial hyphæ studded with yellow granules upon acidfied media. Reverse of colony and substratum (upon these media) colored deep rich red. Surface growth partly of simple conidiophores, partly aerial hyphæ, and ropes of hyphæ (which rarely become vertical coremia) bearing conidiophores as lateral branches. Conidiophores 100–200μ. Conidial fructifications up to 120μ in length, consisting of single verticils of branches 10–16 by 2–2.5μ, bearing whorls of conidiiferous cells 13–15 by 2–2.5μ tapering into acuminate sterigmata bearing conidial chains which are parallel but do not form a column. Conidia elliptical, 3–3.6 by 2μ, smooth, pale green or yellowish green.

Colonies liquefy gelatin, but slowly and incompletely, and give a neutral or acid reaction upon all litmus media. Under different conditions of culture and acidity the discoloration of the medium varies from yellow to orange and deep red. Produces discolorations upon commercial timbers. Habitat, pine wood, which is strongly colored by it.

Culture received from the author, G. G. Hedgcock, of the Forest Pathological Laboratory, Bureau of Plant Industry, United States Department of Agriculture.

Since the publication of his description of this fungus (1906) Hedgcock[9] has reached the conclusion, concurred in by the writer, that this species can not be regarded as identical with the species described by Corda as *P. aureum*. He notes that this species is a common agent in the discoloration of pine wood, hence proposes the name *P. pinophilum* (here first published). Careful consideration of Corda's figure and description would establish a strong presumption that

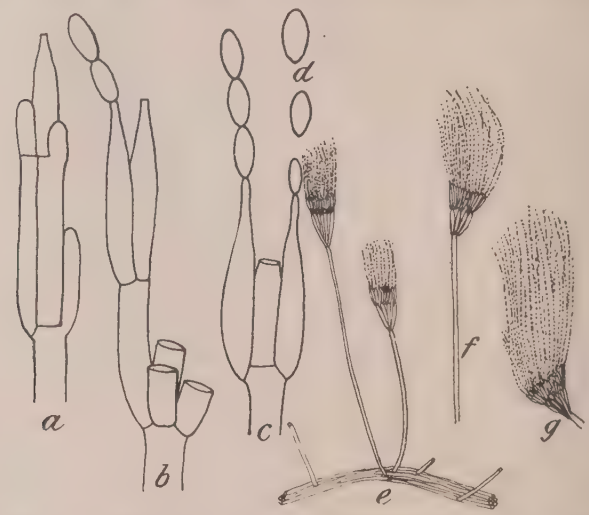

FIG. 6.—*Penicillium pinophilum* Hedgcock: *a*, young conidial fructification showing conidiiferous cells at apex of central branch before all the branches appear (× 1,600); *b*, a verticil of four branches, upon one of which fruit appears (× 1,600); *c*, *d*, conidiiferous cells and conidia (× 1,600); *e*, rope of hyphæ bearing conidiophores sketched (× 140); *f*, *g*, forms of conidial fructification (× 140).

the form described by him would not now be considered a species of *Penicillium*.

CULTURAL DATA.

Color, conidial areas green, vegetative mycelium colorless or studded with yellow granules; reverse of colony red; color in media, red.

Odor, none.

Fifteen per cent gelatin in water, growth slow, surface growth of conidiophores and green conidial fructifications only; liquefaction, none or very slow (in acidified cultures only after several weeks); litmus reaction, acid. Potato agar and bean agar, mycelium studded with yellow granules, conidial areas strict, green; reverse of colony, red. Potato plugs, poor growth, not typical. Cohn's solution, spores germinated only.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, growth fair, hyphæ yellow with granules, acid reaction, conidia green, reverse, red. Lactose, growth slow, not characteristic. Levulose 3 per cent, fair growth, yellow mycelium, acid to litmus. Galactose 3 per cent, fair growth, acid reaction. Glycerin, very slight growth which became typical when sugar was added. Potato starch, good characteristic growth. Butterfat, slow weak growth, with characteristic colors and red fluid.

Milk, growth small; curdling (0.25 per cent calcium chlorid added), very slow; digestion, none or very slight; color in milk, red at top.

At 37° C., grew well, more rapidly than at 20° C.

PENICILLIUM RUBRUM O. Stoll.

Beitr. z. morp. u. biol. char. Penicillium, Würzburg, 1904, p. 35, t. I, fig. 7; t. III, fig. 3; t. IV, fig. 4.

Colonies upon lactose gelatin and potato or bean agar, from green through ochraceous to ochraceous red with varying conditions; consisting of green conidia with yellow mycelium when cane sugar is added. Aerial portion velvety strict or very closely floccose in media without sugar, becoming dense cushions of mycelium bearing successive crops of green conidia in cane-sugar media; marginal growth continuous but slow and not marked by a white border. Reverse and mycelium yellowish to red, yellow in sugar media; the substratum also colored in old agar colonies. Conidiophores arising from substratum directly or as very short lateral branches of the felted hyphæ, mostly 15–30 by 3–3.5μ, swollen at the apex, making a dense layer on the surface. Conidial fructification usually massed into a heavy column with a broad triangular base, 100–200μ in length, from a dense verticil of branches of the conidiophore, each swollen at the apex. Conidiiferous cells 10–13 by 2–3μ, with rather abrupt points from which the conidia are cut off. Conidia at first cylindrical, then elliptical or even globose, 3.4 by 2μ, or 2.5–3.3μ, yellowish green to dark green when mature. Colonies produce slow and only partial liquefaction of sugar gelatin.

A slow-growing fungus fruiting for several weeks and differing greatly in colors with slight and undefined differences in the conditions. Sometimes producing a blood-red color on the reverse of the colony.

Cultures received from Kral. So far not found native in America.

PENICILLIUM LUTEUM Zukal.

Sitzber. K. Akad. Wiss. (Vienna) Math. Naturw. Kl., XCVIII, p. 521, 1889.

Conidial form: Colonies on sugar gelatin and potato or bean agar, white or gray or transiently yellow on media lacking sugars, with sometimes greenish areas of conidial fructification showing shades of yellow (egg yellow) upon sugar media, later passing over to reddish, especially with the formation of aerial wefts or balls of hyphæ producing asci (several weeks); surface rather close floccose, spreading indefinitely upon the substratum. Reverse of colony more or less reddish, especially on sugar media. Conidiophores a thin and incomplete layer, scantily produced mostly as lateral branches of aerial hyphæ, 20–100μ (mostly 30–60) by 3μ. Conidial fructification usually small up to 80μ in length, commonly with a single lateral branch and but two verticils of long acuminate basidia 13–16 by 3–4μ. Conidia elliptical to fusiform, 2.4 by 2.3μ, rather firm walled, greenish, swelling greatly and producing 1 or 2 tubes in germinating.

This species characteristically produces yellow mycelium, from which, in a time varying from a few days upon media rich in sugar to several months upon plain potato agar, ascigerous wefts of hyphæ arise. As given by Wehmer,[29] "ascigerous conceptacles" are 0.5–2 mm. in diameter, globose, vitelline then red; asci reddish, globose to fusiform 8.8 by 7–7.8μ; sporidia 4.8 by 3.3μ, transversely tricostate, hyaline to

reddish. Colonies do not liquefy, or only slowly and partially liquefy, sugar gelatin. Litmus reaction neutral, the shades of purple found about the turning point of litmus. Colonies on apple are brighter yellow and produce ascigerous masses in very few days.

Received from Professor Thaxter, identified from the ascigerous form by Dr. C. Wehmer.

The author is convinced that Wehmer[32] is in error in attributing a large degree of polymorphism to this fungus. Numerous cultures watched under all sorts of conditions are evidence that this species does not produce prominent coremia or large masses of green conidia.

CULTURAL DATA.

Color white, but mycelium studded with yellow granules in acid media, with sometimes reddish areas and conidial green areas; reverse of colony yellow to orange; color in media, pink in potato.

Odor, none.

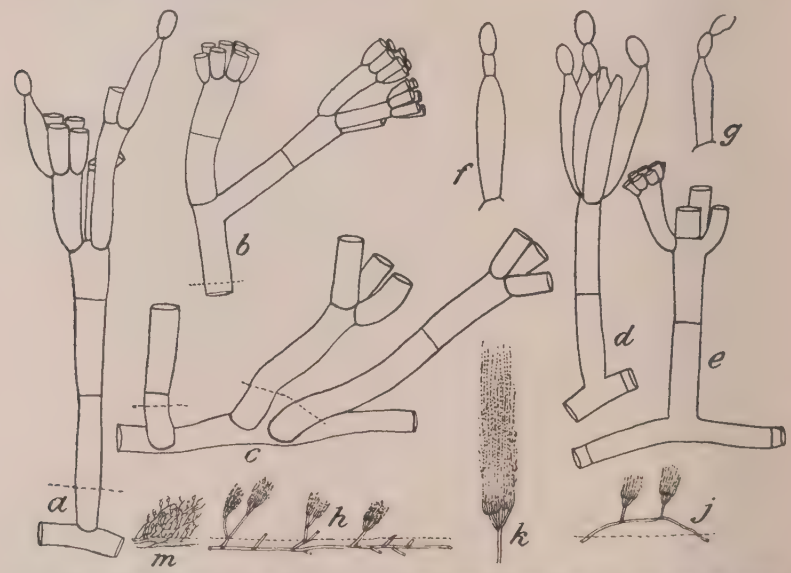

FIG. 7.—*Penicillium rubrum* O. Stoll: *a*, *b*, *c*, *d*, *e*, Whole conidiophores and the branching of conidial fructifications (× 1,400); *f*, *g*, conidiiferous cells and conidial formation (× 1,400); *h*, *j*, sketch and diagram of habit of growth (× 140); *k*, sketches of old conidial fructification in large size (× 140); *m*, diagrammatic figure (the successive series of conidial fructifications are produced by new branches from hyphæ overgrowing the earlier series). (Drawn from gelatin culture, but found with approximately the same morphology upon potato agar.)

Fifteen per cent gelatin in water, medium growth; liquefaction none; litmus reaction acid; potato agar and bean agar, mycelium transiently yellow, then colorless or reddish or yellowish gray; potato plugs, white to gray, in parts yellow, potato pinkish; Raulin's fluid, slow development, bright yellow colonies; Cohn's solution, germination only.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, good growth, yellow, up to 30 per cent sugar, no growth at 50 per cent. Lactose 3 per cent, very slight growth; lactic acid 0.9 per cent, very slight growth. Levulose 3 per cent, very slight growth. Galactose 3 per cent, typical, acid reaction. Glycerin 3 per cent, germination only.

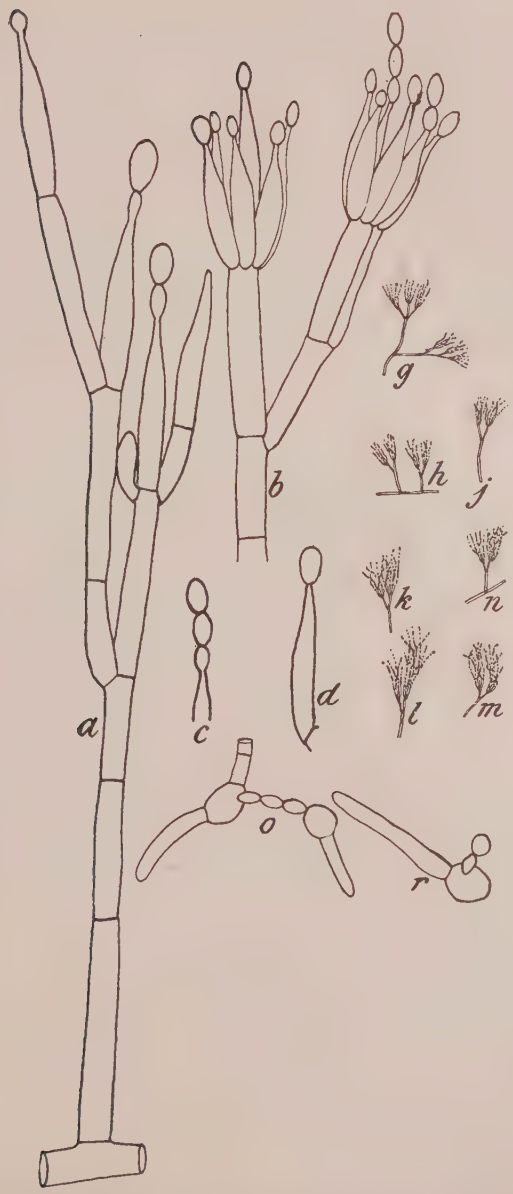

FIG 8.—*Penicillium luteum* Zukal: *a*, whole conidiophore and fructification (× 900); *b*, *c*, *d*, conidiiferous cells, conidia and their arrangement (× 900); *g*, *h*, *j*, *k*, *l*, *m*, *n*, sketches of fructifications (× 140); *o*, *r*, swelling and germination of conidia, swollen conidia in the same chain with those which refuse to grow (× 900).

Potato starch 3 per cent, slow development but characteristic. Butterfat, little or no growth.

Cooked apple, mycelium yellow, ascigerous masses produced most quickly (1 week).

Milk, grows poorly; curdling (with 0.25 per cent calcium chlorid) none; digestion slight and slow.

At 37° C., grew well; at 20° C., more slowly.

PENICILLIUM DUCLAUXI Delacroix.

Bulletin de la Société Mycologique de France, Tome VIII, 1891, p. 107, Pl. VII.

Colonies grown upon gelatin and potato or bean agar clear dark green to olive when old, consisting of short crowded conidiophores arising for the most part singly from the substratum (strict), but sometimes producing short coremia. Long coremia are produced abundantly upon orange, milk, potato, and all media rich in cane sugar. Conidiophores very short, 10–50μ, either arising directly from the substratum or borne

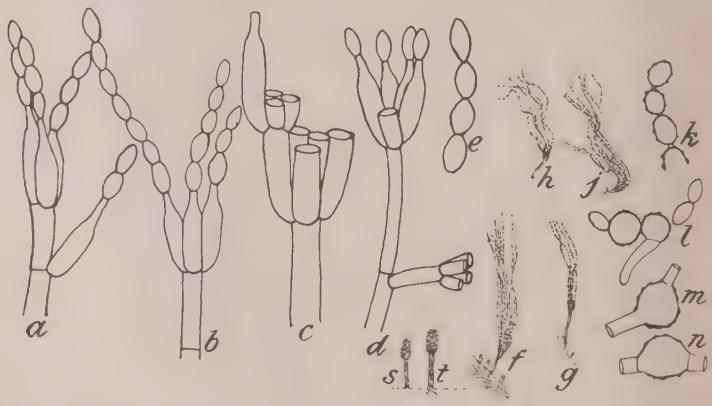

FIG. 9.—*Penicillium duclauxi* Delacroix: *a*, *b*, conidial fructifications with young conidia smooth, from potato-agar plate culture, simpler types (× 900); *c*, *d*, *e*, conidial fructifications from potato-agar plate culture, more complex types (× 1,400); *f*, *g*, *h*, *j*, sketches of habit upon potato agar, showing the very short conidiophores arising from the substratum (× 140); *k*, ripe spores highly magnified to show delicate markings (× 900, apochromatic); *l*, *m*, *n*, germination of conidia (× 900, apochromatic); *s*, *t*, coremia, (sketch).

upon the upper third of the coremia, 1–2 septate, bearing a simple conidial fructification or a terminal fructification and a divergent lateral branch with a whorl of conidiiferous cells. Conidial fructification often 100–160μ in length consisting of a few conidiiferous cells 10–12μ in length in a simple terminal whorl or less commonly in secondary whorls. Conidia elliptical fusiform 3.6–4 by 2–2.5μ, clear homogeneous green, smooth when young, but rugulose when ripe. Colonies liquefy sugar-gelatin in Petri-dish culture slowly from twelfth to twenty-fifth day and change red litmus to blue in 7 days. Produces a coloring agent in sugar media which is wine red in alkaline media and yellow (bile-yellow) in acid media acts as an indicator with neutral point very near that of phenolphthalein).

This fungus is characterized by its enormous development of coremia upon milk, orange, apple, and media containing cane sugar, while producing only very short conidiophores in bean or potato agar and gelatin free from sugar.

Received from the author, George Delacroix, Paris, but one culture previously obtained from P. H. Rolfs in Florida in the summer of 1905 was thought to be this.

This species has not been found commonly in America, but it has been many times observed and recognized as a contamination of other cultures in this laboratory since its introduction. Its identification is therefore easy.

CULTURAL DATA.

Color, fruiting areas olive to clear deep green, often brown when old; reverse, from colorless to yellow and shades of red, according to medium; color in media, none in media free from sugar, in sugar yellow at first, then slowly red (color acts as an indicator—acid when yellow, alkaline when red).

Odor, rather strong in Raulin's fluid, none in most cultures.

Fifteen per cent gelatin in water, medium growth; liquefaction, none in 15 days, then slow in acidified cultures especially; litmus reaction, neutral slowly or weakly alkaline. Potato and bean agar, green fruiting surfaces consisting of very short crowded conidiophores slightly tuberculate at times, but no coremia. Potato plugs, abundant coremia, deep green, reverse and potato yellowish. Raulin's fluid, good growth, many coremia, some odor. Cohn's solution, germination only.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar 3 per cent, rich growth, coremia, poor colonies at 30 per cent and 60 per cent. Lactose 3 per cent, very weak growth, few very small coremia. Lactic acid 0.9 per cent, good growth, typical, coremia many, remains acid. Levulose 3 per cent, weak colonies. Galactose 3 per cent, poor growth. Glycerin 3 per cent, germination and slow, weak colonies. Alcohol, some growth. Potato starch, rich growth, coremia, reverse and fluid yellowish Butterfat, slow but typical coremiform colony, fluid yellow (acid reaction).

FIG. 10.—*Penicillium claviforme* Bainier: *a*, coremium grown upon sugar media, showing branching stalk with several small heads of conidia; *b*, coremium grown upon gelatin free from sugar, showing typical unbranched stalk with a single conidial mass splitting as it increases in size into several columns composed of chains of conidia. (For full illustration of the structure of this species, see Bainier's figures.)

Milk, typical coremiform colonies; curdling (0.25 per cent calcium chlorid added) in 9 days; digestion slow; color, becoming yellow (acid) and later red (alkaline) in very old cultures.

At 37° C. no growth; check grew at 20° C.

PENICILLIUM CLAVIFORME Bainier.

Bulletin Trimestriel de la Société Mycologique de France, Tome XXI, 1905, p. 127, Pl. XI, figs. 8–11; Saccardo, Sylloge Fungorum, Vol. XVIII, p. 520.

Colonies on milk-sugar gelatin and potato agar, white or gray, with surface composed of loosely floccose hyphæ, bearing simple but definitely penicillate fructifications, between the bases of white or yellowish simple or variously branched coremia 1–2 cm. long, fertile only at the apices. Simple fructifications sparingly branched, bearing small verticils of conidiiferous cells 9–10 by 2μ. Coremial fructifications consisting of closely branching and interwoven hyphæ, producing verticils of conidiifer-

ous cells crowded into a false hymenium and producing chains of conidia adhering in olive-green masses 1–3 mm. in height. Conidia elliptical, showing a connective, 4.2–4.6 by 3–3.3 μ, homogeneous green, remaining in chains in fluid mounts. Colonies only partially liquefy gelatin media and give a weak alkaline or neutral reaction with litmus.

Received from G. Bainier October, 1905.

A culture received from Reddick, Ithaca, N. Y., marked Whetzel No. 2095, proved to be this species. It was found at Junius, N. Y. A culture sent to Dr. C. H. Peck was not recognized. Although not closely resembling other species of Penicillium it may best continue under the name given by Bainier until closer affinities are found for it.

CULTURAL DATA.

Color, mycelium white or gray, conidial heads olive green; reverse of colony brown; color in media brownish.

Odor, perceptible in media containing cane sugar, characteristic.

Fifteen per cent gelatin, growth, characteristic coremia, but mainly unbranched; liquefaction slow, partial; litmus neutral. Potato agar and bean agar, typical coremiform colonies. Potato plugs, vigorous typical growth with long coremia. Raulin's fluid, characteristic. Cohn's solution, characteristic.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, typical growth with acid reaction in concentrations from 1.5 to 30 per cent, coremia branching, no coremia at 50 per cent. Lactose 3 per cent, slight growth, poorly nourished colonies, very delicate coremia. Lactic acid 0.9 per cent, germination only. Levulose 3 per cent, slow-growing, poorly nourished colony. Galactose 3 per cent, typical growth, acid reaction. Glycerin 3 per cent, not typical, no coremia, coremia produced after addition of cane sugar. Malic acid 1 per cent, slight growth, few and very small coremia. Butterfat, typical colonies.

Milk, good characteristic growth; curdling (0.25 per cent calcium chlorid added) in 9 days; digestion slow but fairly complete; color in milk brown or reddish.

At 37° C., no growth in 6 days; culture grew when cooled to 20° C.

PENICILLIUM GRANULATUM Bainier.

Bul. Soc. Mycol. France, XXI, 1905, p. 127, Pl. XI, figs. 6, 7.

Colonies upon plain gelatin and potato or bean agar yellowish green to gray or grayish brown, superficially composed of crowded small coremia 1–3 mm. in height, mixed with floccose hyphæ and separate conidiophores, spreading indeterminately upon the substratum. Reverse reddish orange (approaching "fulvous"), aerial hyphæ delicately granular or spinulose, which separates this from all other species studied. Conidiophores 4–4.5μ in diameter, short or very long, either separate or, mostly, massed into very short, crowded coremia (less than 1 mm. in height). Conidial fructifications usually 100–200μ in length, once or twice verticillate, with many conidiiferous cells 9 by 2–2.5μ, and long, loosely divergent chains of conidia. Conidia at first cylindrical, then elliptical to globose, about 2.5–3 by 3–3.5 or 3μ in diameter, yellowish green, granular, remaining in long chains in fluid mounts. Colonies do not liquefy gelatin, litmus reaction slowly alkaline.

The delicately granular or spinulose hyphæ as noted and figured by Bainier are a valid and distinctive character. The species is also easily recognized by its general appearance and habit. Obtained from the type cultures of Professor Bainier, Paris.

CULTURAL DATA.

Color yellowish green to green and later various shades of brown; reverse of colony orange, or yellow to deep orange, or even red; color in media yellow to orange to red in media containing starch or sugar.

Odor, none.

Fifteen per cent gelatin in water, characteristic growth; liquefaction, none, or partial after several weeks in acidified cultures; litmus reaction, strongly alkaline. Potato agar and bean agar, yellow-green to brown, yellow below, coremia not closely crowded as in sugar media. Potato plugs, characteristic growth, potato stained yellow to deep brownish yellow. Raulin's fluid, characteristic growth. Cohn's solution, germination only.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, 3–30 per cent, characteristic growth and colors, but not normal at 60 per cent, with acid reaction. Lactose 3 per cent, characteristic structure but weak development. Lactic acid 0.9 per cent, growth, but not normal colonies. Levulose 3 per cent, slow development, small colonies. Galactose 3 per cent, characteristic. Glycerin 3 per cent, slight growth, grew freely when cane sugar was added. Potato starch, characteristic. Butterfat, typical growth, reverse and fluid orange; yellowish.

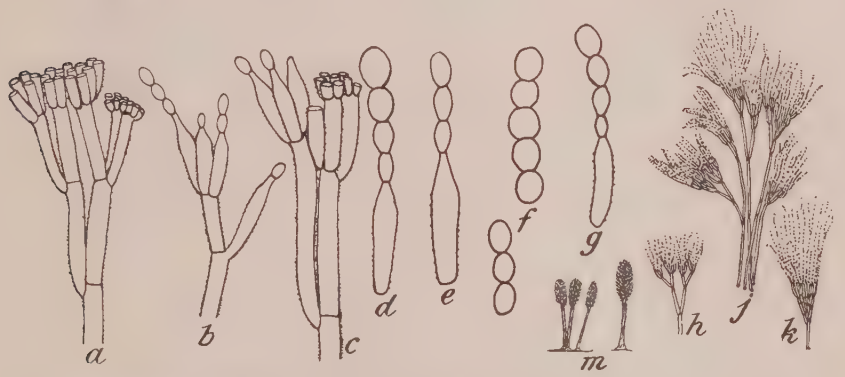

FIG. 11.—*Penicillium granulatum* Bainier: *a, b, c,* branching of conidial fructification showing granulated echinulated cell walls (× 900); *d, e, f, g,* conidiiferous cells and conidial chains (× 1,400); *h, j, k,* sketches of fructifications (× 140); *m,* sketches drawn from photomicrograph of coremia showing fertile and sterile areas (× about 25).

Milk, rapid and characteristic growth; curdling, none; digestion, slow but complete (fairly); color in milk, yellow to orange to deep red.

At 37° C., no growth, conidia grew when removed to lower temperature; at 20° C., excellent growth.

PENICILLIUM BREVICAULE Saccardo.

Fungi Italici, No. 893, Mich., II, p. 547.

Colonies grown upon sugar gelatin grayish white, then yellowish brown or chocolate, consisting of short closely crowded conidiophores making powdery areas overgrown by loosely trailing floccose hyphæ and ropes of hyphæ, with broadly spreading indeterminate margin. Conidiophores, short, 10–30μ mostly, arising directly from the submerged hyphæ, or numerously and irregularly borne as lateral and perpendicular branches of trailing aerial hyphæ and ropes of hyphæ. Conidial fructifications either simple chains terminating unbranched or sparingly branched conidiophores in young colonies, or verticillately and irregularly twice verticillately branching systems bearing numerous divergent chains often 150μ in length in old colonies. Conidiiferous

cells continuous with conidiophores 12–15 by 4μ tapering to slender sterigmata. Conidia somewhat pear-shaped, slightly tuberculate at apex, with broad base, 6.5–7.5 by 7.5–9μ, in mass light brown to chocolate; at first smooth, then with thick tuberculate walls, viable for many months, germinating by a single tube from the thin center of the broad base into a bulbous enlargement from which mycelial hyphæ about 2μ in diameter arise. Mycelium very thin walled, narrow cells of varying length. Colonies liquefy sugar gelatin and give a strong blue reaction in litmus media, but grow very tardily, if at all, in potato or bean agar. Grows very rapidly upon neutral or alkaline media, but very slowly or not at all in media acid to litmus. Digests milk. Refused to grow after repeated inoculation into sterilized apple. Spores which refused to germinate in the agar media used grew immediately when transferred to any of the gelatin media.

Cosmopolitan, forms characteristic chocolate patches on Camembert cheese. Secured from numerous brands of cheese and common in the laboratories of this station.

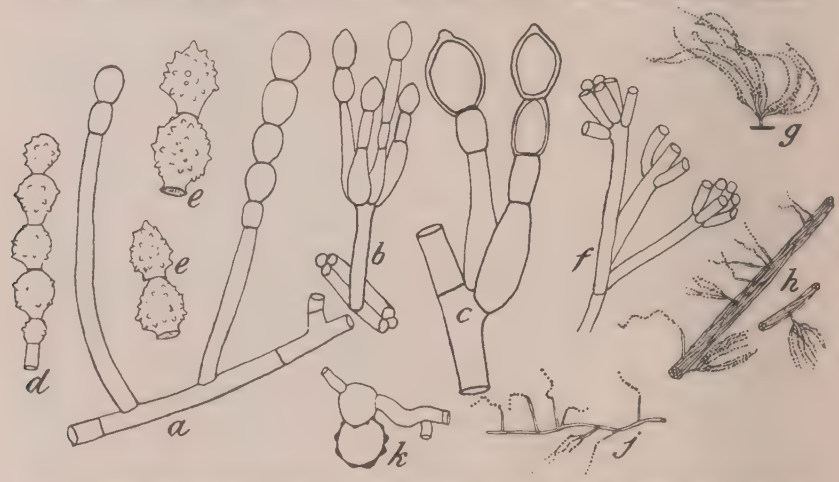

FIG. 12.—*Penicillium brevicaule* Saccardo: *a*, conidiophores and simple conidial chains with spores still smooth (× 900); *b*, *f*, more complex conidial fructifications (× 900); *c*, two young conidial chains, showing thick walls of spores (× 1,400); *d*, *e*, conidia after becoming echinulate (× 1,400); *g*, *h*, *j*, sketches of forms and habit of conidial fructifications (× 140); *g* from an old cluture, sessile or almost so; *h* and *j* show trailing hyphæ and a rope of hyphæ with lateral conidiophores; *k*, germinated conidium where the old spore wall lies empty beside the growing cell (× 1,400).

The author does not believe that this species is closely related to other species of this genus, but since it has been placed here by a very liberal interpretation of descriptions it may perhaps remain under this name until someone finds out its real affinities. The two forms which follow as varieties are found in the same habitat, show closely similar morphology, and give almost identical physiological reactions. Their designation as varieties of *P. brevicaule* may therefore be justified, though one at least (var. *glabrum*) seems separate enough to warrant proposing for it a specific name.

CULTURAL DATA.

Color, conidial surfaces clay-yellow to chocolate; reverse of colony, mycelium colorless; color in media, none.

Odor, ammoniacal, used as a test for arsenic (Gosio et al.).

Fifteen per cent gelatin in water, typical colony; liquefaction, rapid 5–6 days; litmus, strongly alkaline. Prefers alkaline, neutral, or only slightly acid media. Potato agar, spores sometimes refuse even to germinate, but grow when transferred to gelatin; grows poorly on agar media free from sugar, or peptone, or the by-products of other fungus growth. Common as a secondary growth in such plates. Potato plugs, good colonies. Cohn's solution, characteristic but slow growth. Raulin's fluid, germinated, but very slight growth.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar in agar 3 per cent, failed to grow, same spores transferred to gelatin media grew promptly. Lactose 10 per cent, slow, characteristic color to conidia. Lactic acid 0.9 per cent, germination only. Levulose 2.5 per cent, growth characteristic, alkaline reaction to litmus. Galactose 3 per cent, characteristic growth, alkaline reaction to litmus. Glycerin 3 per cent, spores germinated only, grew well upon addition of cane sugar. Potato starch grew well. Malic acid, germinated only. Butter fat, slow development, finally producing drops of yellow oil which separate out.

Milk, rapid growth; curdling (0.25 per cent calcium chlorid added) in 10 days; digestion, rapid; color in milk, none.

Cooked apple, failed to grow after repeated inoculation.

At 37° and 20° C., grew equally well.

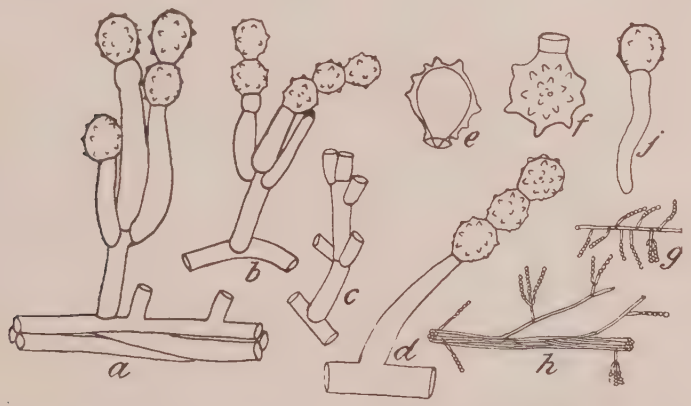

FIG. 13.—*Penicillum brevicaule*, var. *album: a, b, c, d,* conidiophores and fructification borne variously and differently branching (× 900); *e, f,* ripe conidia; *g, h,* sketch of single hyphæ and a rope of hyphæ bearing conidiophores (× 140); *j,* germinated spore.

PENICILLIUM BREVICAULE Saccardo, var. ALBUM Thom, n. var.

Colonies upon sugar gelatin white to cream-colored alike above and below upon all media, strict to sparsely floccose, with trailing hyphæ and ropes of hyphæ, indeterminately spreading. Conidiophores either arising from substratum directly or mostly as perpendicular branches of ærial hyphæ and ropes of hyphæ 15–40μ in length, conidial fructification varying from a single chain to more or less complex penicillate branching, mostly producing few chains of indefinite length and arrangement from narrow tapering conidiiferous cells. Conidia pyriform to subglobose, with basal collar, 9–10μ, roughly tuberculate, white or slightly yellowish tinged, thick walled except at the base, the center of which remains as a germ pore. Colonies rapidly liquefy sugar gelatin with strong ammoniacal odor, and give an intensely alkaline reaction in litmus media. Gives exactly the same reactions as *P. brevicaule* Sacc. Differs from the latter slightly, except in the color of the spores.

Common upon imported Camembert cheese. Found often upon domestic Camembert and grows very readily in cheese cellars, where it becomes a nuisance.

CULTURAL DATA.

Same as *P. brevicaule* Sacc., except the following difference noted:

Conidia cream, somewhat larger than *P. brevicaule*.

Cohn's solution failed to produce characteristic colonies.

In Dox's solution, with butterfat as a source of carbon, it differs from *P. brevicaule* by failing to cause drops of yellow oil to separate out.

Agar-agar: In repeated cultures this organism has failed to grow well in agar media. In some such cases the spores transferred from the agar to gelatin grew at once. Some cultures upon agar grow slowly and in typical manner, but the development upon all agar media has seemed uncertain. In synthetic solution cultures were obtained when the inoculation of tubes of the same solution with 1.5 per cent of agar added to make a solid substratum produced no growth.

PENICILLIUM BREVICAULE Saccardo, var. GLABRUM Thom, n. var.

Colonies white or only slightly yellowish-tinged in all gelatin media, grow not at all or with difficulty on agar of most formulæ. Aerial portion consisting of short, closely crowded conidiophores making a powdery surface overgrown by loosely trailing hyphæ and ropes of hyphæ, spreading broadly over the substratum. Conidiophores, short, mostly 10–30μ, arising directly from submerged hyphæ or numerously and irregu-

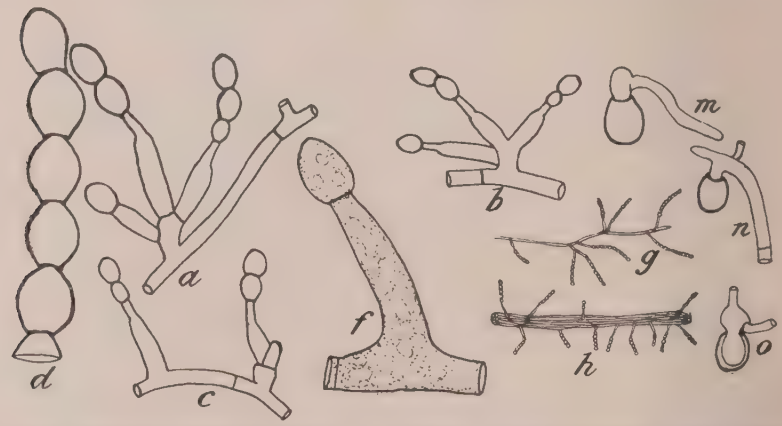

FIG. 14.—*Penicillium brevicaule*, var. *glabrum*: *a*, *b*, *c*, branching of conidial fructification (× 900); *d*, chain of conidia (× 1,400); *f*, formation of conidium on young branch (× 1,400); *g*, *h*, sketch of appearance in culture (× 140); *m*, *n*, *o*, germination of conidia.

larly borne as perpendicular branches of the superficial hyphæ and ropes of hyphæ. Conidial fructifications from simple chains of spores to fairly complex penicillate groups of branchlets resembling *P. brevicaule*, but mostly less complex. Conidia obovate, pyriform 7–8 by 8–10μ or almost globose, 7–9μ, smooth, white, rather thick-walled and retaining their power to germinate for many months. In old potato and other cultures black sclerotia are formed in the substratum but do not produce asci. Liquefies gelatin rapidly (within one week), gives a strong alkaline reaction and ammoniacal odor.

Habitat, found repeatedly on imported Camembert cheese and secondarily upon domestic soft cheese, where it grows into prominent cottony patches indistinguishable to the eye from the white variety of *P. brevicaule*. This fungus is separated from *P. brevicaule* by its smooth white spores and the production of the black sclerotia in the substratum.

This form is certainly closely related to *P. brevicaule* by its physiological reactions and its general morphology. It was found in one case among the exsiccati in the Harvard herbarium under the name of *Monilia candida*. There is, however, no possibility of confusing this form with that species as understood and described by more recent students, such as Hansen and Jorgenson.

CULTURAL DATA.

Exactly as in *P. brevicaule*, except for the following:

Conidia smooth, somewhat smaller.

Color more nearly white.

Sclerotia black, found in very old potato-plug cultures or in agar cultures which have grown several weeks or months.

Cohn's solution failed to produce a characteristic colony.

PENICILLIUM ROSEUM Link (?).

Colonies on milk-sugar gelatin or potato agar white to pink or salmon in fruiting areas, loose floccose with simple hyphæ and ropes of hyphæ, producing dense irregular

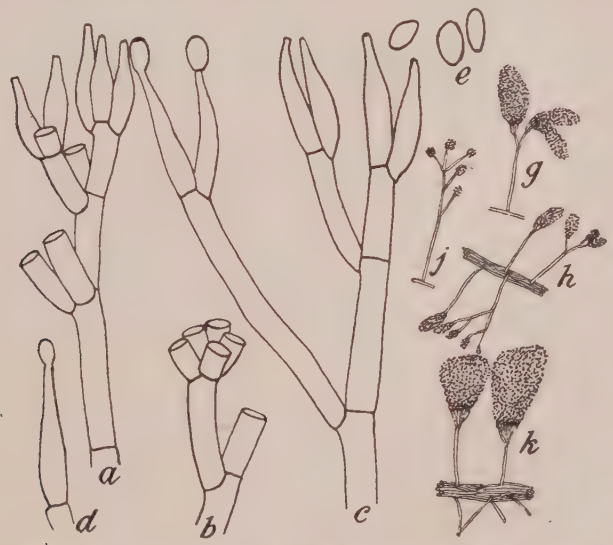

FIG. 15.—*Penicillium roseum* Link (?): *a*, *b*, *c*, branching of conidial fructification, showing few cells in each verticil (× 900); *d*, *e*, conidiiferous cell and conidia (× 900); *g*, *h*, *j*, *k*, sketches of ripe fructifications showing agglutination of conidia into slimy masses (× 140).

pinkish masses or sclerotia up to 1 mm. or more in diameter in old culture. Conidiophores borne as perpendicular branches of aerial hyphæ or ropes of hyphæ, 45–125μ. Conidial fructification up to 140μ in length, once or twice irregularly alternately or verticillately branched, with conidiiferous cells varying from 12 by 2–3μ in the verticils of 5 or less to 17 by 2.3μ when solitary, bearing conidia which become aggregated into gelatinous balls or masses. Conidia colorless (pink or rosy in mass), elliptical, 5–7 by 3–5μ, slightly apiculate, smooth, appearing delicately granular within. Colonies liquefy gelatin cultures rapidly and give an alkaline reaction to litmus media.

Brought from Kral, in Prague, Bohemia.

The same organism has been found once in accidental culture in this laboratory; received once from a correspondent in Halle, Germany, and later found under this name as No. 1179 in De Thümen's Mycotheca Universalis; collected by Ravenel in South Carolina in 1876 upon leaves of *Buxus;* this and several other specimens were found in the mycological collection of the Bureau of Plant Industry, United States Department of Agriculture. The spores are the same length as given by Saccardo, but slightly broader. The number of specimens found under this name from widely different workers appears to justify the belief that this is the organism described by Link under this name. If the development of a mucilaginous mass enveloping the conidia be regarded as a sufficient basis for separation of such species under the generic name of *Gliocladium,* this species would become *Gliocladium roseum* (Link).

The form upon *Buxus* is cited by Saccardo, referring to it as "*P. roseum* Cooke, non-Link," and held to be *Verticillium buxi* Auersw. et Fleisch. Examination of the material would indicate that in De Thümen's collection at least this species is more closely allied to the other species of *Penicillium* than to *Verticillium.*

CULTURAL DATA.

Color white or shades of salmon pink; reverse cream or white; color in media, none.

Odor, none.

Fifteen per cent gelatin in water, medium growth, white. Liquefaction, rapid. Litmus reaction, alkaline, strongly. Potato agar and bean agar, good growth, but white. Potato plugs, white colonies. Cohn's solution, slight growth.

In Dox's solution, with butterfat as a source of carbon, this species caused drops of yellow oil to separate out.

At 37° C., killed; check at 20° C., good.

PENICILLIUM CAMEMBERTI Thom.

Emended from U. S. Department of Agriculture, Bureau of Animal Industry, Bul. 82, p. 33, fig. 1, 1906.

Possible syn.: *P. album* Epstein (not Preuss), Archiv f. Hyg., Bd. 45, Hft. 4, p. 360, 1902.
P. epsteini Lindau, Deutschl. Krypt. Flora; Pilze, VIII, p. 166.

Colonies on potato agar or lactose gelatin effused; white (sometimes yellowish white), changing in 5–8 days to gray-green (glaucous); surface of colony floccose, of loosely felted hyphæ about 5μ in diameter, reverse of colony yellowish white; conidiophores 300–800μ in length, 3–4μ in diameter, septate, cells thin-walled, often collapsing in age, arising as branches of aerial hyphæ; fructification sometimes 175μ in length, but usually much less, consisting commonly of one main branch and one lateral branch, sparingly branched to produce rather few conidiiferous cells which bear long loosely divergent chains of conidia. Conidiiferous cells 8–11 by 2.4–3μ. Conidia at first cylindrical, then elliptical, and finally globose when ripe, smooth bluish green by transmitted light, thin-walled and commonly guttulate, 4.5–5.5μ in diameter, swelling in germination to 8–10μ. Germ tubes one to several. Cells of mycelium about 5 by 20–40μ. Liquefies lactose gelatin only under center of colony. Produces a strong

alkaline reaction in gelatin, free from sugar, but in sugar media produces a more or less persistent acid reaction. Growing and fruiting period, about 2 weeks. Fruits only upon exposed surfaces of the substrata; never produces spores in cavities not broadly open.

Habitat, Camembert and other soft cheeses.

The name *P. album* Epstein, which is also *P. epsteini* Lindau,[10] is inserted in the list of possible synonymy because this name is accepted for this mold by Mazé [13] in a recent paper. If we base identification upon the cultural characters given for his mold by Epstein, it could not have been *P. camemberti*. The characterization, so far as given by Epstein and extracted from his paper by Lindau, might refer to the pure white form (*P. candidum*) as interpreted by Roger and Mazé better than to this species. There is certainly at least a varietal difference (spore color) between *P. camemberti* and the *P. candidum* of Roger and Mazé, which is designated a variety of *P. camemberti* in this paper.

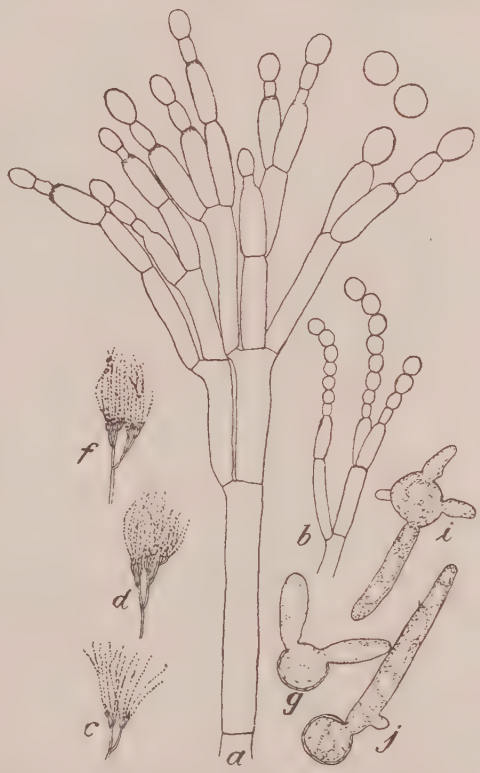

FIG. 16.—*Penicillium camemberti* Thom: *a*, conidiophore showing a common type of branching and the production of basida and conidia, highly magnified; *b*, a common form showing much less branching; *c*, *d*, *f*, diagrams of large fructifications (× 80); *g*, *i*, *j*, germinating conidia. (From Bull. 82, Bureau of Animal Industry.)

The change in litmus reaction in this description from that previously published is result of proof that no acidity is produced by this species except when sugar is present. A change from blue to red and black to blue indicates the production of acidity by fermentation of sugar followed by the neutralization of the acid so produced by the alkaline by-products of proteolysis or by a further change in the acid. Therefore this statement is best omitted from the diagnostic description.

CULTURAL DATA.

Color white, through cream to a shade of gray-green upon sugar media, without sugar gray or drab, all becoming drab when old (exposure to light); reverse of colony, uncolored, cream; media, uncolored.

Odor, none, except in very old cultures upon milk or cereal.

Fifteen per cent gelatin in water, moderate growth, not rich; liquefaction, none, or slow and partial after 2 to 3 weeks in acidified cultures; litmus, strongly alkaline; colonies slow, moderate growth, typical. Potato agar and bean agar and potato plugs, fair, white to gray colonies lacking or nearly free from green color. Raulin's fluid, very heavy growth, white through pronounced cream to green shades. Cohn's solution, slow growth, characteristic, but lacking in green color.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, characteristic, abundant green color in solutions as high as 50 per cent, acid. Lactose 3 per cent, characteristic, acid reaction. Lactic acid 0.9 per cent, slowly typical, with ultimately an alkaline reaction to litmus. Levulose 3 per cent, slowly typical. Galactose 3 per cent, characteristic growth, very slowly developing the typical green color. Alcohol about 5 per cent, characteristic. Potato starch, characteristic. Malic acid 1 per cent, fair colony, colorless. Butterfat, slowly typical colony with a tinge of violet in reverse, no color in fluid.

Milk, typical growth; curdling (0.25 per cent calcium chlorid added) in 10 days; digestion, slow but fairly complete; color in milk, none.

Camembert cheese, capable of reducing moist curd soured by lactic organisms to a semiliquid condition.

At 35° to 37° C., no growth; at 20° C., excellent.

PENICILLIUM CAMEMBERTI, var. ROGERI Thom, n. var.

Syn. *P. candidum* of Roger and Mazé, not Link.

Colonies grown upon sugar gelatin or bean or potato agar pure white, loosely and evenly floccose to the very margin, where aerial and submerged hyphæ grow with equal rapidity. Reverse of colonies white or yellowish white (not discolored). Conidiophores 3–5 by 100–800μ varying greatly, mostly branches of aerial hyphæ. Conidial fructification 70–90μ in length, loosely and irregularly branched and bearing rather few basidia at unequal heights, with divergent chains of colorless conidia. Branching system of conidial fructification sometimes 75μ in length. Conidia smooth, hyaline, 4–4.5 or even 5.5μ in diameter, globose or nearly so when ripe. Sugar gelatin is slowly liquefied under the center of the colony only, colonies never floating in a pool of liquid. Reaction in the medium is acid to litmus at first, then changes to alkaline.

This fungus has been found by the author only upon Camembert, Brie, and Neufchâtel cheeses from western Europe.

This variety has been discussed by Mazé [13] as *P. candidum* Link. This seems an impossible application of the name *P. candidum* from Link's description [12] or that given by Saccardo, since the spores are stated to be 2–3μ in diameter. Further, in such identifications no account is taken of a paper by Morini,[16] in which an ascigerous stage is described for *P. candidum* Link. Under four years of cultivation no signs of an ascigerous form have been produced. Stoll [24] has considered *P. candidum* to be only a colorless *P. glaucum*, but as the author has so far failed to find a worker who will undertake to limit the name

P. glaucum to a special form, this does not mend matters. Long cultivation does show, however, that this organism is closely related to the one already described as *P. camemberti*.[25] Since this is the form given prominence in cheese studies by the work of Georges Roger,[21] it seems most natural to regard it as a variety of the former species and designate it by Roger's name.

In Lafar's Handbuch der Technischen Mykologie, Professors Weigmann[36] and Wehmer[34] refer to this fungus as probably iden-

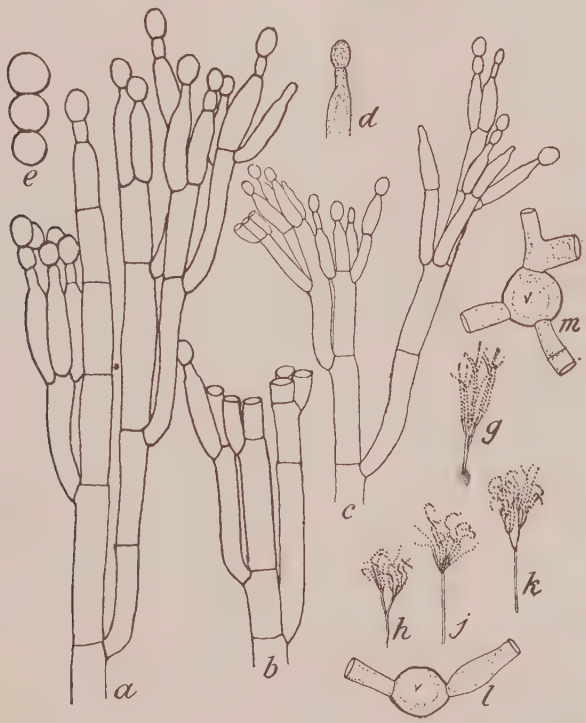

FIG. 17.—*Penicillium camemberti*, var. *rogeri:* *a*, *b*, *c*, types of branching of conidial fructification (× 900); *e*, ripe conidia, showing variation in size (× 900); *g*, *h*, *j*, *k*, sketches of conidial fructifications (× 140); *l*, *m*, germination of conidia by several tubes.

tical with *P. camemberti*. However this may appear from examination of the literature alone, no one actually familiar with the cultures will claim such identity. If the possession of colorless spores be regarded as a case of albinism, this form may perhaps be regarded as a variety of *P. camemberti*. It has been kept separate and remained constant in culture for several years. It would seem, therefore, equally proper to regard it as a different species were it not so closely associated with *P. camemberti* in every other character.

The name *P. candidum* was used by Link for a mold growing upon decaying leaves, bulbs, and fungi, which was said to be common, and Morini [16] later describes an ascigerous form of this fungus. The spores are of different size (2–3μ). There appears to be no justification for adopting this name for the fungus used by Roger in cheese making.

CULTURAL DATA.

Different from *P. camemberti* only as follows:

Color of conidia persistently white.

Cohn's solution, failed to germinate. Same spores transferred to gelatin after 4 months grew normally.

Camembert cheese, does not produce the same texture as the preceding species.

PENICILLIUM BIFORME n. sp.

Latin diagnosis.[a]—Coloniis in gelatina cultis, albis, lente glaucescentibus, densius floccosis, margine sterili lata, aut, in agaro solani tuberosi cultis, albis glaucescentibus, mox avellaneis vel fere olivaceis, parte aeria ex conidiophoris brevissimis et creberrimis fructibus conidicisque composita; conidiophoris (sine ramis) 60–150μ in agaro, vel longioribus ramosis ex hyphis floccosis in gelatina cultis; fructibus conidicis 100–200μ longis, plerumque 1–2 ramosis alternates, ramis convergentibus vel divergentibus, ramulis verticillatis basidia apice verticillata gerentibus; basidiis 8–10 usque $13 \times 3\mu$, conidiis primum ellipticis vel cylindricis demum globosis, $3.2–3.5 \times 4–4.3$, vel 4μ diam., in catenis manentibus submersis; coloniis copiosis in saccharo lactis, gelatinam in parte lente liquefacientibus, alkalinis lacmo, odore mucidis.

Habitat, in caseo, ex Gallia.

Cultivated in gelatin, white, slowly gray-green, densely floccose, with broad vegetative margin, spreading widely over the substratum; in potato agar white, then gray-green, rapidly becoming yellowish-brown, drab, or almost olive, restricted in growth, aerial portion consisting of very short densely crowded conidiophores and conidial fructifications; conidiophores 60–150μ on agar or slightly longer when arising as branches from the floccose aerial mycelium growing upon gelatin; conidial fructifications mostly once or twice alternately branched, branches convergent or divergent, each bearing a verticil of branchlets crowned by verticils of conidiiferous cells with chains of conidia, the whole 60–240μ, usually 100–200μ, in length; conidiiferous cells 8–10 or even 13 by 3μ; conidia elliptical or cylindrical, then globose, 3.2–3.5 by 4–4.3μ or 4μ in diameter, adhering in chains in fluid mounts; grows luxuriantly in fluid offering milk-sugar as source of carbon, partially and slowly liquefies gelatin, with alkaline reaction to litmus; odor, very strong, "moldy," characteristic.

This species was obtained from cheese sent from France by Georges Roger. It was afterwards obtained from other French cheeses, but does not seem to have any economic importance. It is closely related by cultural characters as well as morphology to *P. camemberti*, with which it shares the ability to grow normally in fluid offering lactose as the source of carbon, but differs in its short conidiophores, diverse habit upon potato agar and gelatin, and its strong charac-

[a] The author is indebted to Prof. H. R. Monteith, of the Connecticut Agricultural College, for much assistance in preparing the Latin diagnoses.

teristic smell. It is perhaps intermediate in character between *P. camemberti* and the group of forms designated as *P. commune* in this paper.

CULTURAL DATA.

Color, white to gray-green, gray, or drab in some media, all brown or drab when old; reverse cream; color in media, none.

Odor, very strong peculiar "moldy" odor, typical of this species under nearly all conditions.

FIG. 18.—*Penicillium biforme: a, b, g,* branching of conidial fructification from potato-agar culture (× 1,400); *c, d, e, f,* conidiiferous cells and conidia (× 1,400); *h, j, k,* sketches of conidial fructifications on potato agar (× 140); *l, m,* sketches of conidial fructifications on sugar gelatin (× 140); *o, r,* germination of conidia (× 900).

Fifteen per cent gelatin in water, typical floccose colony; liquefaction, partial in old acidified cultures, none in 15 days; litmus reaction, strongly alkaline (in nearly all media). Potato agar and bean agar, colonies consisting only of very short conidiophores green to drab in color, little or no floccose mycelium. Potato plugs, typical. Cohn's solution, slow, half normal growth, with odor.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, typical up to 50 per cent sugar, with acid reaction. Lactose 3 per cent, rich growth, typical. Lactic acid 0.9

per cent, typical, alkaline reaction when old, crystal drops. Levulose 3 per cent, typical. Galactose 3 per cent, slow development, typical. Glycerin 3 per cent, typical. Potato starch 3 per cent, typical colony, crystal transpiration drops. Butter-fat, typical colony, mycelial mass tinged violet.

Milk, rich growth; curdling (0.25 per cent calcium chlorid added), in 8 days; digestion, rather slow; color in milk, none.

At 37° C., no growth, slowly green when cooled; check at 20 ° C., typical.

PENICILLIUM COMMUNE n. sp.

In examining numerous Petri-dish cultures made for the examination of milk by the bacteriologists of the Storrs Experiment Station, large numbers of colonies of *Penicillium* have been studied. A very large percentage of these colonies have a series of common characters which are constant enough to mark out a species, or, perhaps better, a group of races, between which differences are either minute or so complicated by the occurrence of other races with overlapping characters as to make their separation a matter of considerable doubt. One of these has been selected as the basis of the following diagnosis. This form is morphologically closely similar to *P. expansum* (see figs. 1 and 19). It, however, lacks entirely the ability to form coremia and fails to discolor the substrata, but grows well in certain culture solutions which markedly restrain *P. expansum*, of which it might possibly be regarded as a variety. From its abundance in the situations studied it has been designated as *P. commune.*

Latin diagnosis.—Coloniis in gelatina vel agaro Solani tuberosi aut phaseoli cultis, viridibus, demum brunneolis, in substrato late crescentibus, zonatis; marginis crescentis parte aeria ex conidiophoris, centri atque ex hyphis plus minusve floccosis composita; reverso et substrato incolorato; conidiophoris plerumque 300μ raro usque 700μ longis; fructibus conidicis 100–200μ longis, cum ramis alternatis et verticillatis confertis, basidiis 8–9×3μ cum apicibus brevibus acutis, catenas conidiorum longas parallelis gerentibus; conidiis primum cylindricis vel ellipticis, demum globosis, 3–4μ diam., 5–6μ incrassatis germinantibus, levibus, viridibus, in catenis manentibus submersis; coloniis in gelatina in parte lente liquefacientibus; odore mucidis.

Habitat in lacte, caseo, etc., Storrs, Conn.

Colonies in gelatin or in potato or bean agar, dull green, becoming brown when old, broadly spreading, zonate, with broad white growing margin composed only of conidiophores, in the older parts becoming floccose masses of interwoven hyphæ; reverse of colony and substratum never colored; conidiophores commonly 300μ or less in length, sometimes up to 700μ; conidial fructifications commonly 100–200μ in length, compact at base and broadening above, variously branching with branches appressed, and verticils of conidiiferous cells 8–9 by 3μ, abruptly narrowed to produce conidia; conidia cylindrical to elliptical and finally globose 3–4μ, becoming 4–5μ or larger in germinating, smooth, green, persisting in chains in fluid mounts; colonies liquefy gelatin slowly or partially, softening rather than producing clear liquid, alkaline in media without sugar but acid with either cane sugar or lactose, having a strong "moldy" odor.

Habitat, common in food, dairy products, etc., Storrs, Conn.

CULTURAL DATA.

Color green, becoming brown when old; reverse cream or white; color in media, none.

Odor, strong, "moldy."

Fifteen per cent gelatin in water, good growth; liquefaction, none in 15 days, slow or partial in older cultures; litmus reaction, slowly alkaline. Potato agar and bean agar, characteristic. Potato plugs, characteristic. Raulin's fluid, characteristic, with odor "moldy." Cohn's solution, small colonies, trace of pink below.

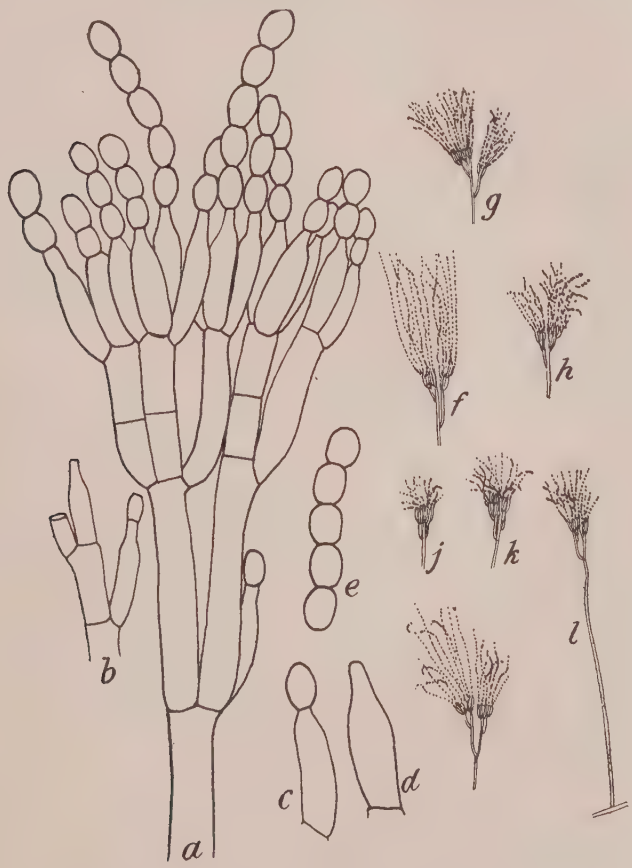

FIG. 19.—*Penicillium commune:* *a, b, c, d, e,* conidial fructification, branching, and production of conidia (× 900); *f, g, h, j, k, l,* sketches of fructifications in various stages (× 140).

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, typical culture, transpired drops crystal. Lactose 3 per cent, typical culture. Lactic acid 0.9 per cent, good growth. Levulose 3 per cent, good culture, strong odor. Galactose 3 per cent, good culture, strong odor, alkaline reaction. Glycerin 3 per cent, typical, strong odor. Butter fat, typical growth.

Milk, good typical colony; curdling (0.25 per cent calcium chlorid added) in 1 week; digestion, slow; color in milk, none.

At 37° C., no growth, grew upon cooling; check at 20° C., rich growth.

PENICILLIUM No. 22.

Colonies in gelatin or agar gray-green or glaucous persistently, or becoming gray, not brown, otherwise appearing as *P. commune;* conidial fructifications more loosely branching, with branches divergent; conidia larger and lighter color; odor, none or indefinite; reactions as in *P. commune.*

Habitat: Isolated several times from domestic soft cheeses made in the State of New York, 1904-5; found associated with *P. camemberti;* in appearance and color resembling latter species, but in structure of colony, measurements of conidiophores, etc., resembling *P. commune.* In pure culture this form has maintained its identity clearly for four years.

CULTURAL DATA.

Color gray-green; reverse colorless; color in media, none.

Odor, none or very slight.

Fifteen per cent gelatin in water, good growth; liquefaction, none in 15 days, partial in 2 to 3 weeks or more; litmus reaction, blue. Potato agar and bean agar, good typical colonies, with alkaline reaction to litmus. Potato plugs, good growth, typical gray-green colony with crystal drops of exuded water. Cohn's solution, medium growth, trace of pink in media.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, good growth up to concentration of 60 per cent, acid reaction. Lactose 3–10 per cent, typical. Levulose 3 per cent, good growth. Galactose 3 per cent, good growth, typical. Glycerin 3 per cent, good growth. Butter fat, rich growth, typical.

Milk, typical colonies; curdling (0.25 per cent calcium chlorid added) in 8 days; digestion, rather slow; color in milk, none.

At 37° C., no growth, grew when cooled; check at 20° C., typical.

PENICILLIUM CHRYSOGENUM n. sp.

Latin diagnosis.—Coloniis in gelatina vel agaro Solani tuberosi aut phaseoli cultis, griseo-viridibus, demum brunneolis, in substrato late crescentibus, margine sterili lata juvenilibus parte aeria ex conidiophoris et caespitibus sparsis hyphorum adscendentium composita; reverso incolorato; conidiophoris plerumque singulatim usque $300 \times 4\mu$ orientibus, raro brevibus ex hyphis assurgentibus ramosis; fructibus conidicis 100–200 longis cum 1–2 ramis alternatis et divergentibus ramulos 1–2 verticillatos gerentibus; basidiis $8 \times 2.5\mu$ verticillatis ex apicibus ramulorum, catenas divergentes conidiorum gerentibus; conidiis primum cylindricis vel ellipticis, demum globosis, 3–4 diam., pallide glaucis, magnis vacuolis; coloniis gelatinam liquefacientibus, alkalinis lacmo; lactem, panem, gelatinam, auream colorantibus.

Habitat, in caseo, pane, etc., commune.

Cultivated in gelatin, or bean or potato agar, gray-green, becoming brownish when old; aerial portion consisting of conidiophores with some tufts of trailing aerial hyphæ; broadly spreading in the substratum with a wide sterile margin when young. Reverse of colony not discolored. Conidiophores mostly arising separately, up to 300 by 4μ, partly arising as short branches of aerial hyphæ; conidial fructifications 100–200μ in length, with 1–2 alternate divergent branches, bearing alternate, verticillate or twice verticillate branchlets. Conidiiferous cells 8 by 2.5μ verticillate at the ends of branchlets bearing divergent chains of conidia. Conidia cylindrical or elliptical at first, then globose, 3–4μ in diameter, pale green, with large vacuoles. Colonies liquefy gelatin, with alkaline reaction to litmus and produce in milk, bread, gelatin, and other substances a golden yellow color (from which the name).

Habitat, in bread, cheese, etc., apparently common and appearing in several varieties which differ in the intensity of color production, in appearance in certain cultures, but which are so far scarcely distinguishable by structural characters.

The culture here named *P. chrysogenum* has been kept under observation for more than four years without change. In this time several forms have been collected or sent to this laboratory which also produce the golden color in the digestion of milk suggestive of the name proposed. The cultural characters of these races differ in some degree in several cases, in others substantial identity has been observed. In reporting cultural data three numbers are included for comparison with this, viz, Nos. 25, 35, and 44. These agree in the following characters: Spreading habit, surface mostly

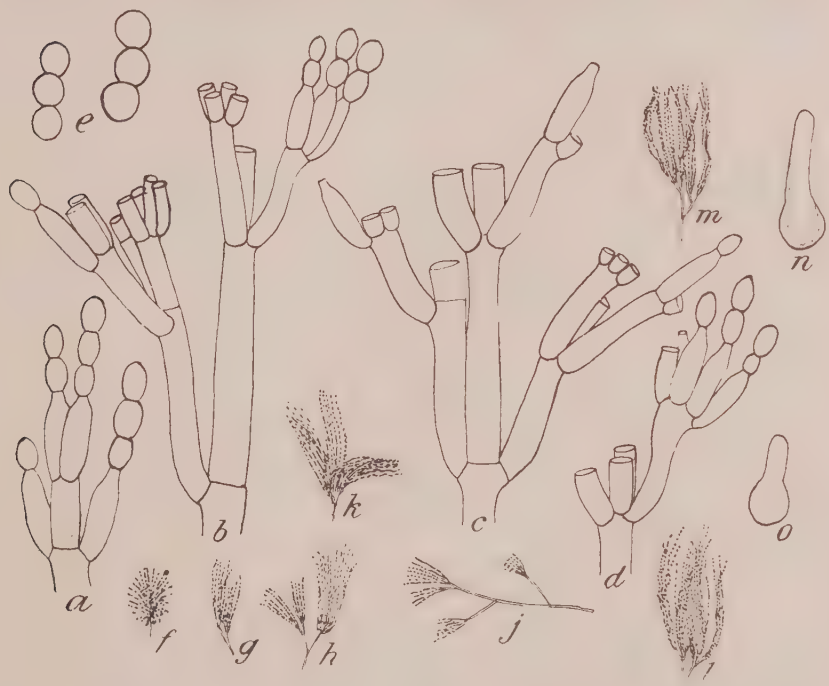

FIG. 20.—*Penicillium chrysogenum: a, b, c, d, e,* branching of conidial fructification from gelatin plates (× 900); *f, g, h, j, l, m,* sketches of conidial fructifications from potato-agar plates (× 140); *n, o,* germination of conidia (×900).

of conidiophores averaging about 300μ in length, nearly the same shade of green in color, similar morphology of conidial fructification, digestion of milk, gelatin, etc., with the production of yellow or golden color, liquefaction of gelatin progressive but slower than the expansion of the colony in the substratum so that a growing border of submerged vegetative hyphæ extends into hard gelatin for considerable time, in contrast to forms in which the colony becomes a floating "island" in a pool of fluid within the first week. In spite of these common characters colonies of these four races often show

great apparent differences in parallel culture in some specialized media. It seems preferable to let this name stand in a broad enough sense to include the forms having the common characters above noted than to attempt any narrower delimitation of this group of forms at this time.

Serial No. 57.—Another form which may be included temporarily with this group does not produce a yellow color in the substratum at all, but produces mycelium of orange color as seen from below. The surface growth is clear green without definite differences from the *chrysogenum* group, but the orange color in reverse remains as a constant difference from that group, unattended by any coloration of the medium.

CULTURAL DATA.

(Cf. Nos. 25, 35, and 44 in tables.)

Color gray-green, green, to brown when old; reverse colorless, or slight yellowish. Color in media golden yellow in certain media, no color in others.

Odor, none.

Fifteen per cent gelatin in water, good growth, yellow color in gelatin; liquefaction rapid—6–10 days, more or less rapid and complete in all gelatin media used; litmus reaction, alkaline. Potato agar and bean agar, good colonies, but no yellow color. Potato plugs, typical, potato becoming yellow. Raulin's fluid, typical with yellow transpired drops, but no yellow in fluid. Cohn's solution, growth, but not normal.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, good growth up to 30 per cent, reaction acid, fluid colorless. Lactose 3 per cent, fair growth, not heavy, fluid yellow, with acid reaction. Lactic acid 0.9 per cent, some growth. Galactose 3 per cent, good growth. Glycerin 3 per cent, colony white to green, no yellow color in fluid. Potato starch 3 per cent, good growth, yellow drops above, yellow in fluid. Butter fat, rich growth, with fluid golden yellow.

Milk, rich growth; curdling (0.25 per cent calcium chlorid added) in 1 week; digestion rapid; color yellow.

PENICILLIUM RUGULOSUM n. sp.

Latin diagnosis.—Coloniis in gelatina vel agaro phaseoli cultis, flavo-viridibus, dein viridibus, demum atro-viridibus, late crescentibus in agaro; parte aeria ex conidiophoris creberrimis et hyphis aereis et paucis composita; reverso luteo et in parte aurantiaco imprimis in tubere Solani; conidiophoris 100–200×2.5–3μ, singulatim vel ex hyphis aereis prope substratum orientibus; fructibus conidicis 100–150μ longis (in saccharo multo longioribus) ex ramis 10–15×2.5μ, compacte verticillatis, verticillos basidiorum, vel ramulorum, vel ramulorum et basidiorum eodem verticillo gerentibus; basidiis 9–12×2μ, acuminatis, catenas longas et divergentes conidiorum gerentibus; conidiis 3.4–3.8×2.5–3μ, ellipticis, viridibus, uno apice incrassato, verruculosis maturis, in catenis manentibus submersis, 5μ diam. incrassatis germinantibus; coloniis non (vel solum in parte et lente) gelatinam liquefacientibus.

Commune in culturis, Storrs, Conn.

Cultivated in gelatin or bean agar, yellowish green, then green, at length dark green; surface growth of densely crowded conidiophores with few aerial hyphæ interspersed at their bases; reverse of colonies yellow to orange in spots, especially upon potato or upon sugar media; substratum not or slightly yellowed; conidiophores 100–200 by 2.5–3μ, arising separately or branching from aerial hyphæ just above the substratum; conidial fructifications 100–150μ in length, consisting of appressed, verticillate branches

10–15 by 2.5μ, bearing verticils of conidiiferous cells, of branchlets, or of conidiiferous cells and branchlets together; conidiiferous cells 9–12 by 2μ, acuminate, bearing long divergent chains of conidia; conidia 3.4–3.8 by 2.5–3μ, elliptical, green, mostly with swelling at one end, verruculose when ripe, swelling to 5μ and germinating by one or two tubes; colonies do not or only partially liquefy gelatin.

Common in cultures, Storrs, Conn. Characterized by its verrucose spores and the brilliant color of the mycelium viewed from below.

CULTURAL DATA.

Color green or yellowish green; reverse yellow to orange or reddish orange; color in media, none.

Fifteen per cent gelatin in water, slow development; liquefaction, none or slight in old colonies; litmus reaction neutral. Potato agar, good growth, alkaline. Bean agar + 5 per cent cane sugar, rich heavy growth producing conidia in heavy dark-green masses, easily broken off by shaking the tube. Potato plugs, green, reverse bright orange.

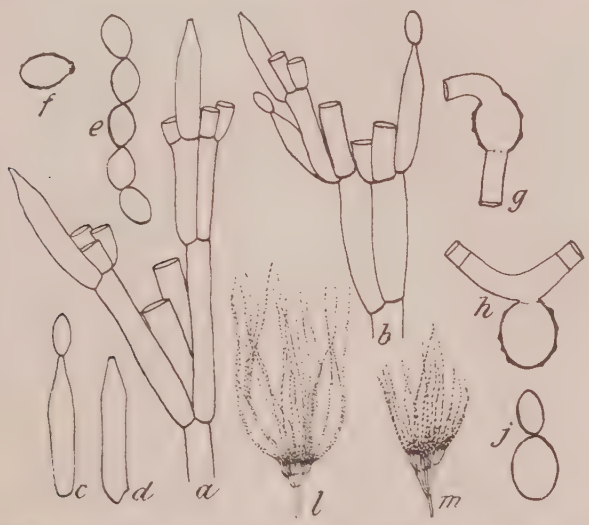

FIG. 21.—*Penicillium rugulosum*: *a*, *b*, branching of conidiophore (×1,600); *c*, *d*, *e*, conidiiferous cells (basidia) and conidia (× 1,600); *f*, fully ripe conidium, showing delicate roughening of walls (× 1,600); *g*, *h*, *j*, swelling and germination of conidia (× 1,600); *l*, *m*, diagrams of conidial fructifications (× 260).

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, growth in concentration up to 60 per cent, acid. Lactose, 10 per cent, slight growth. Levulose, fair growth, acid reaction; galactose, good growth; glycerin, slow growth; butterfat, slowly typical colony, yellow below; fluid, slightly yellowish.

Milk, growth typical, reverse yellow to orange or reddish orange; curdling (0.25 per cent calcium chlorid added) in 9 days; digestion slow or slight; color, none in milk.

At 37° C., no growth, grew when cooled; check at 20° C., typical.

PENICILLIUM CITRINUM n. sp.

Latin diagnosis.—Coloniis in gelatina vel agaro Solani tuberosi aut phaseoli cultis, aeruginoso-viridibus, demum fuligineis; fructibus viridibus usque ad marginem gestis, i. e. margine sterile angustissima; coloniis in gelatina rotundis, parvis, cito liquefa-

centibus; in agaro latioribus; parte aeria ex conidiophoris et fructibus conidicis creberrimis composita, interdum caespitibus paucis hyphorum adscendentum in medio; reverso incolorato; conidiophoris (sine ramis) non longioribus 150μ, singulatim orientibus, aut paucis ex hyphis adscendentibus ramosis; fructibus conidicis 3–5 ramorum 16–30×3μ, apice 5μ incrassatorum, in verticillo, basidia in verticillis compactis gerentum; utroque verticillo catenis conidiorum in columno compacto 50–150μ longos adhaerentibus; basidiis 6–7×2–3μ; conidiis globosis, 2.4–3μ raro 3.5μ diam., aeruginoso-glaucis, granulatis intus, in catenis manentibus submersis.

Coloniis, saccharo commixto, substrata citrina in colore efficientibus (ubi nomen).

Habitat, in caseo, pane, etc., commune (?).

Colonies grown upon gelatin and potato or bean agar blue-green when young, becoming dark brown when old, with colored fruit borne almost to the very margin

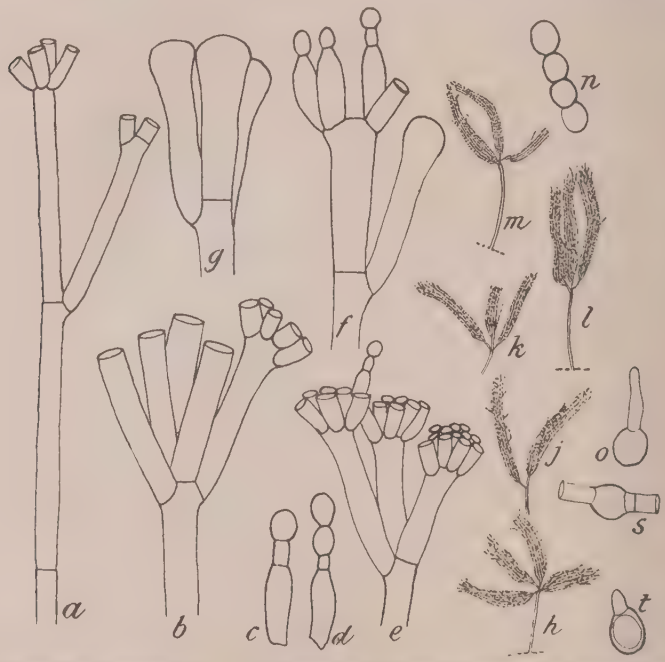

FIG. 22.—*Penicillium citrinum*: *a, b, e, f, g*, branching of conidial fructification, showing number of branches in each verticil and enlargement of ends of branches (*a* × 900, others × 1,600); *c, d, n*, conidiiferous cells and the formation of conidia (× 1,600); *h, j, k, l, m*, sketches of conidial fructifications (× 140); *o, s, t*, germination of spores (× 900).

of the colony, so that the white border of submerged mycelium and uncolored fruit is very narrow; restricted in growth to a few millimeters in diameter upon gelatin, but becoming much larger upon agar; aerial part of colony consisting of densely standing conidiophores and conidia except in the center, where there arise a few tufts of trailing aerial hyphæ. Reverse of colony itself colorless or only yellowish. Conidiophores arising separately, rarely longer than 150μ, branching acropetally from submerged hyphæ radiating from the center of the colony, or branched from the hyphæ of the central aerial tuft. Conidial fructification a verticil 2 to 5 branches 16–30 by 3μ enlarged at apex to 5μ, each producing a compact verticil of conidiiferous cells bearing chains of conidia massed together into columns 50–150μ in length (usually 80–100μ).

The whole fructification appears in this way double, triple, or quadruple or even more complex by a secondary verticil from the central branch. Conidiferous cells 6–7 by 2–3. Conidia globose when ripe, 2.4 to 3.6 diam. in cane-sugar cultures, in diameter, bluish-green, slightly granular in contents, adhering in chains in fluid mounts, losing vitality rapidly with change of color in old colonies. Colonies liquefy gelatin rapidly, so that they lie in pools of liquid within a week. Litmus reaction in plain gelatin strongly alkaline. Produces a lemon-yellow color soluble in alcohol in media containing sugars, milk, gelatin, bread, and potato.

Found in cultures from milk and cheese, probably cosmopolitan.

Could this be *P. citreo-nigrum* Dierckx?

CULTURAL DATA.

Color bluish green, becoming dark brown when old if exposed to light; reverse colorless or yellowish; color in media lemon-yellow when cane sugar or gelatin or peptone is present, none in some media.

Odor, none.

Fifteen per cent gelatin in water, characteristic growth, becoming white by secondary sterile mycelium; liquefaction rapid, colonies floating in 5–6 days; litmus reaction alkaline. Potato agar and bean agar, colonies spreading more than upon gelatin, agar not colored or slightly colored. Potato plugs, typical growth, potato colored yellow, with yellow drops transferred. Raulin's fluid, typical growth, very slight yellow color. Cohn's solution, good growth, no yellow color.

Synthetic fluid (Fox's, carbon supplied as cane sugar, good growth with yellow color up to 30 per cent, with acid reaction. Lactose 3 per cent, slow poor growth giving a violet ringe instead of yellow color. Lactic acid 0.3 per cent, slight growth. Lactose 3 per cent, small characteristic growth. Galactose 3 per cent, good growth, reaction neutral or slightly acid. Glycerin 3 per cent, very small colonies, no color in medium. Alcohol, 3 per cent, good growth, no yellow. Potato starch 3 per cent, good growth, slight if any yellow. Tartaric acid, slow colorless colony. Butterfat, rich growth, lemon-yellow fluid.

Milk, rapid growth; curdling (0.2% per cent cadmium chlorid added) in 10 days; digestion rapid; coloration pale yellowish.

At 37° C., slow growth, white colony, no color in medium; check at 20° C., rich growth, green, yellow in medium, in bean agar with cane sugar.

PENICILLIUM No. 37.

(Var. of *P. citrinum*? Or allied to *P. citrinum*?)

Colonies in media without sugar, green, gray-green, or gray; with sugar persistently green; surface velvety sorid, composed of short crowded conidiophores up to 100μ in length, branching from closely woven mycelium, partly submerged, partly aerial, with margin narrow, not widely spreading in the substratum; reverse of colony and substratum not colored in many; conidial fructifications sometimes a single verticil of conidiferous cells, sometimes 2 to 4 verticillate branches; chains of conidia from each verticil forming a column up to 50–60μ in length in sugar media; branches of fructification 10–14 by 2–2.5 enlarged at apex; conidiferous cells 8–10 by 2–4 abruptly narrowed into sterigmata, usually 6–10 in each verticil; conidia broadly elliptical to globose, 2.7–3.6 at first becoming 4–5. Before germinating, thin-walled, smooth, pale yellowish green, germinating by a single tube; colonies liquefy gelatin rapidly (6 to 7 days), with strong alkaline reaction to litmus.

Received from Prof. P. H. Rolfs, Miami, Fla., in culture upon bean stems, 1905.

Allied to *P. citrinum* by morphology and culture reactions, but differing in lacking the power to color media yellow and in its greater dependence for typical growth upon the presence of cane sugar.

CULTURAL DATA.

Color light blue-green, olive, or gray in various media; reverse, white or cream; color in media, none or slightly yellowish.

Odor, none.

Fifteen per cent gelatin in water, small olive-green colonies; liquefaction, rapid—6 to 7 days; litmus reaction, alkaline. Potato agar and bean agar, typical. Potato plugs, typical. Raulin's fluid, typical. Cohn's solution, slow and weak-growing colony.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, good growth even up to 60 per cent, acid reaction. Lactose, 3 per cent, slow abnormal growth. Lactic acid, 0.9 per cent, small but characteristic colony. Levulose, 3 per cent, small colo-

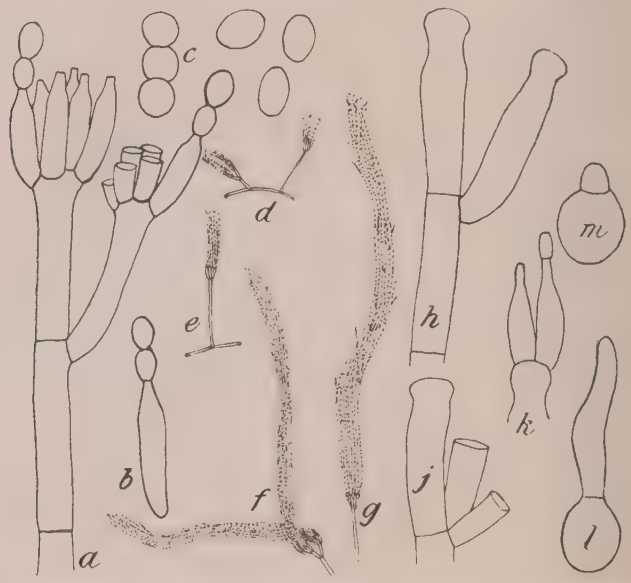

FIG. 23.—*Penicillium* No. 37: *a*, typical branched fructification, two verticils of conidiiferous cells (× 1,600); *b*, *c*, conidiiferous cell and conidia (× 1,600); *d*, *e*, sketches of conidial fructifications from potato-agar culture (× 140); *f*, *g*, sketches from an old culture on 3 per cent cane-sugar agar, showing simple (*g*) and branched (*f*) forms (× 140); *h*, *j*, *k*, branching of conidiophore, swollen ends of branches (× 1,600); *l*, *m*, germination of conidia (× 1,600).

nies. Galactose, 3 per cent, typical. Glycerin, 3 per cent, small growth. Potato starch, typical. Butterfat, slow growth, deep heavy green colony.

Milk, curdling (0.25 per cent calcium chlorid added), rapid;[a] digestion rapid and complete; color, none.

At 37° C., killed; check at 20° C., grew well.

PENICILLIUM No. 12.

This form differs from *P. citrinum* in producing no coloration of the medium and in producing conidial fructifications in which the chains of conidia are more or less divergent instead of aggregated into columns. In culture there is general corre-

[a] The time of curdling is almost impossible to determine in cases where digestion begins quickly and progresses rapidly.

spondence in reactions, without identity; this form appears to be much more dependent upon cane sugar for the production of typical color of the conidia and growth than is *P. citrinum*. There is also a greater tendency to the production of a layer of mycelial hyphæ just above the surface of the substratum, from which the conidiophores arise as aerial branches.

This form was received from Prof. C. E. Marshall, Agricultural College, Michigan under the name of *P. glaucum*.

CULTURAL DATA.

Color pale blue-green; reverse of colony cream, not colored; color in media, none. Odor, none.

Fifteen per cent gelatin in water, rather small pale blue colonies, rapidly becoming white by secondary sterile growth of hyphæ; liquefaction, rapid—6 days; litmus reaction, alkaline. Potato agar, as in gelatin. Potato plugs, very poor growth, grayish or yellowish green. Raulin's fluid, slow but typical colonies, delicate blue; Cohn's solution, slow and restricted growth.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, grows well in concentrations up to 60 per cent. Lactose 3 per cent, small colonies lacking nourishment. Lactic acid 0.9 per cent, small colonies floating in fluid. Levulose 3 per cent, good growth, alkaline reaction. Galactose 3 per cent, good growth, alkaline reaction. Glycerin 3 per cent, slow-growing colonies, becoming gray-brown when old. Potato starch, good colonies. Butterfat, slow and ill-nourished growth.

Milk, rapid growth; curdling (0.25 per cent calcium chlorid added) in 7 days; digestion rapid and very complete; color in milk, none.

At 37° C., killed in 6 days; at 20° C., good growth.

PENICILLIUM ATRAMENTOSUM n. sp.

Latin diagnosis.—Coloniis in gelatina vel agaro Solani tuberosi aut phaseoli cultis, viridibus, parte aeria plerumque ex conidiophoris singulatim orientibus, medio cum hyphis aereis interspersis, margine albo ex hyphis fertilibus angusta; reverso incolorato vel parum ochraceo; substrato aut incolorato aut in substratis saccharinis et in lacte atrobrunneo tarde fere atro; conidiophoris 240–300 usque 400μ longis; fructibus conidicis 100 usque 200μ longis, ramis 1–2 verticillatis 2–4 inæqualiter longis in verticillo in apice incrassatis; basidiis 8–10μ longis, parallelis in verticillo; catenis conidiorum eodem verticillo in columno compactis; conidiis ellipticis, 3.5–4 (usque 4.8) $\times$2.5–3 usque 3.5μ, lævibus, viridibus, 6–7μ incrassatis et uno tubo germinantibus; coloniis gelatinam cito liquefacientibus, alkalinis lacmo; odore in lacte proprio, in substratis aliis nullo.

Ex caseo cultum, Storrs, Conn., 1905.

Affine P. citrino.

Colonies upon gelatin or upon potato or bean agar bright green, aerial part mostly of simple conidiophores, mixed in older parts with branching aerial hyphæ but narrowly spreading at the margin by new conidiophores only. Reverse of colonies shows a slight production of yellow (ochraceous) color. Conidiophores 240–400μ, averaging about 300μ in length. Conidial fructification up to 200μ in length, usually 100μ or less, verticillately or twice verticillately branched; branches 2–4 in a verticil divergent, unequal in length, swollen at ends, bearing conidiiferous cells. The conidial chains from each verticil form a dense column, which diverges more or less from the other columns when old. Conidiiferous cells 8–10μ in length, closely parallel. Conidia elliptical, varying from 3.5–4μ by 2.5–3μ on agar, somewhat larger in gelatin cultures, up to 4.8 by 3.5μ, smooth, homogeneous green with a slight yellowish shade when seen in mass, swelling to 6–7μ in diameter and germinating by a single tube. Mycelial cells 5–7μ in diameter and up to 30μ or more in length. Colonies liquefy sugar-gelatin

rapidly, give an alkaline reaction to litmus, digest milk, and color potato agar containing high percentage of sugar a deep black.

Found upon Camembert cheese imported from France. Closely related morphologically to *P. citrinum*, from which it is separated by the longer conidiophores and larger spores as well as the black discoloration of sugar media.

CULTURAL DATA.

Color deep (blue) green to brown when old; reverse uncolored, or brown in some media; color in media, none, or brownish to almost black.

Odor, none.

Fifteen per cent gelatin in water, deep green, brown when old, rich growth; liquefaction, 7 days, varies from 6 to 12 days in other gelatin media; litmus reaction, alkaline. Potato agar and bean agar, typical, no color below. Potato plugs, dark green, potato blackened. Raulin's fluid, rather weak growth. Cohn's solution, germinated only.

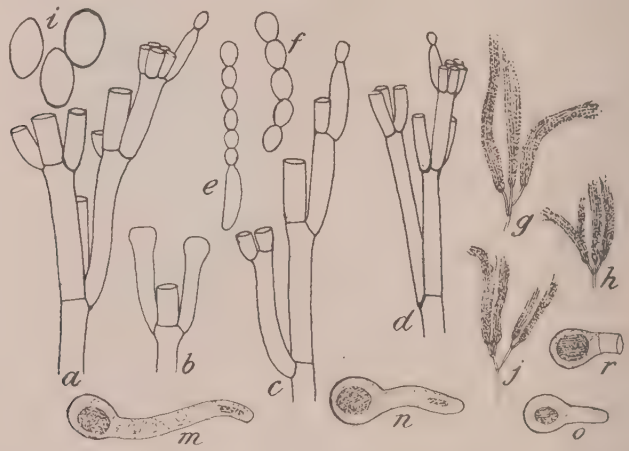

FIG. 24.—*Penicillium atramentosum*: *a*, *b*, *c*, *d*, branching of conidial fructification showing unequal length of branches, swollen ends (× 900); *e*, *f*, conidiiferous cell and chain of conidia (× 900); *g*, *h*, *j*, sketches of conidial fructifications (× 140); *i*, conidia (× 1,600); *m*, *n*, *o*, *r*, germination of conidia (× 900).

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, good growth up to 50 per cent, with acid reaction in 50 per cent solution. Lactose 3 per cent, small and slow growth. Lactic acid 0.9 per cent, no growth. Levulose 3 per cent, slowly typical. Galactose 3 per cent, typical, with alkaline reaction. Glycerin 3 per cent, germination. Potato starch 3 per cent, good growth, no color in fluid or reverse of colony. Butterfat, typical, green colonies with reverse brown, and fluid uncolored.

Milk, curdling (0.25 per cent calcium chlorid added) in 9 days; digestion, rapid, fairly complete; color, brownish to almost black.

At 37° C., no growth, grew when cooled; check at 20° C., typical.

PENICILLIUM No. 24.

(Related to *P. atramentosum*?)

Colonies upon gelatin and potato or bean agar blue-green, becoming brown rapidly when old, or smoky with very dense velvety surface consisting of conidiophores arising in the substratum or just above its surface, with a very abrupt narrow white

margin of unripened fruit and submerged mycelium during the growing period. Reverse of colony and medium colorless under all conditions studied. Conidiophores from 100 to 400μ, averaging about 250μ, in length, either arising separately or as lateral branches of hyphæ just above the substratum. Conidial fructification up to 200μ in length produced by various branching from the conidiophores in which each primary branch is often divergent to produce separate mass of conidia. Conidiiferous cells 7–10 by 3μ. Conidia globose 3.3–4μ, homogeneous blue-green, smooth, seeming to lose vitality rapidly under laboratory conditions. Colonies liquefy gelatin in 7 to 12 days so that they lie in pools of liquid. Litmus reaction strongly alkaline.

Found in the cultures from Camembert cheese in the laboratories at Storrs, Conn. Differs from the preceding and from *P. citrinum* by its longer conidiophores, the alternate branching of its fructification, the size of its spores, and by failure to color the substrata. The relative value of ellipticity of conidia as a diagnostic character appears to be questionable. This form is therefore presented under cultural number

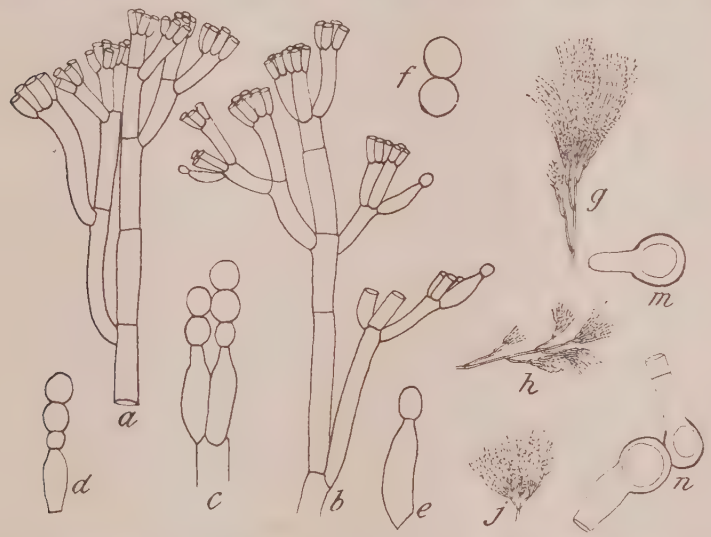

FIG. 25.—*Penicillium* No. 24: *a*, *b*, branching and arrangement of branches in conidial fructification (× 140); *c*, *d*, *e*, conidiiferous cells and conidia (× 1,400); *g*, *h*, *j*, sketches of form and arrangement of conidiophores (× 140); *m*, *n*, germination of conidia (× 900).

only, whereas the preceding has been identified from accidental cultures more frequently, and hence is given name and description as a species.

CULTURAL DATA.

Color deep green (blue-green), becoming brown when old and exposed; reverse white or cream; color in media, none.

Odor, none.

Fifteen per cent gelatin in water, characteristic colony; liquefaction, rapid—11 days or even less; litmus reaction alkaline. Potato plugs, deep blue-green, crystal drops. Raulin's fluid, weak but characteristic growth. Cohn's solution, slow but characteristic development.

Synthetic fluid (Dox's), carbon supplied: Cane sugar, good growth up to 30 per cent, acid reaction. Lactose, 3 per cent, slow development, not typical. Lactic acid 0.9 per cent, good colony. Levulose 3 per cent, small colonies. Galactose 3

per cent, good growth, strongly alkaline. Glycerin 3 per cent, weak growth. Potato starch, characteristic colony.

Milk, curdling (0.25 per cent calcium chlorid added) in 8 days; digestion medium rapid; color in milk, none or ?

At 37° C., no growth, grew when cooled; check at 20° C., good typical.

PENICILLIUM STOLONIFERUM n. sp.

Latin diagnosis.—Coloniis in gelatina vel agaro Solani tuberosi cultis, viridibus vel flavo-viridibus, demum griseo-viridibus vel griseis in agaro sine saccharo, cum sac-

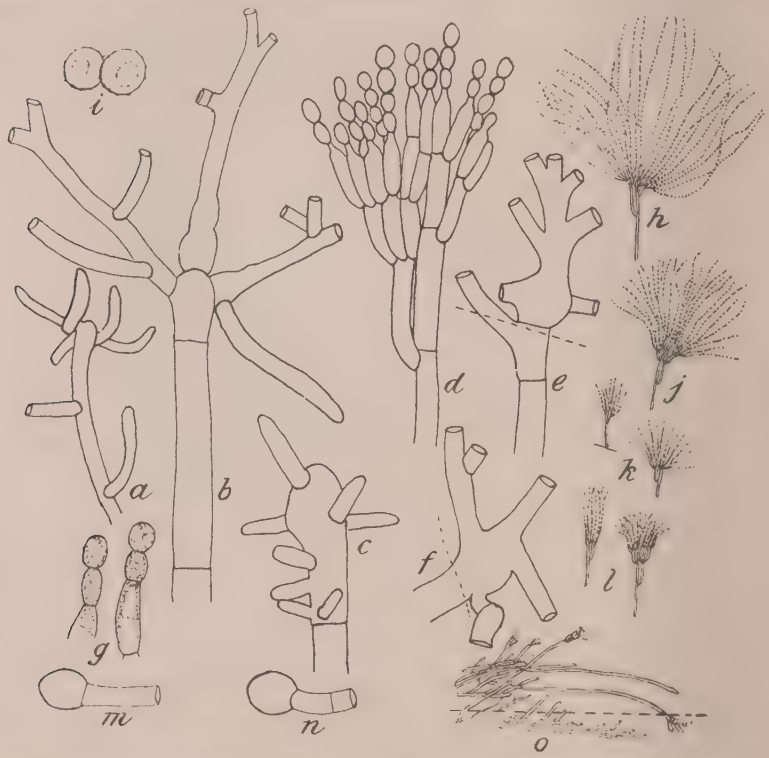

FIG. 26.—*Penicillium stoloniferum: a, b, c, e, f*, the types of branching at the tips of the "stolons" by which this species spreads in substrata (*b, c, e, f*, × 900); *d*, conidial fructification (× 900); *h, j, k, l*, sketches of conidial fructifications of various ages, *h* and *j* being characteristic shapes (× 140); *g*, formation of conidia (× 900), *i*, ripe conidia showing minute granulation (× 1,600); *m, n*, germination of conidia (× 900); *o*, rough diagram of habit.

charo viridibus, floccosis, in culturis juvenalibus stolonibus aereis citius quam hyphis submersis crescentibus, reverso incolorato vel in parte flavo; conidiophoris brevibus ex hyphis adscendentibus ramosis, usque 100μ longis, aut singulatim orientibus (sine ramis) plus minus 300μ longis, fructibus conidicis 40–80μ raro usque 170μ longis, ex ramis brevibus compactis, et basidiis verticillatis, in baside confertissimis, catenas conidiorum late divergentes gerentibus compositis, interdum ramus infimus tam divergens ut fructus duplex videatur; basidiis 10× 3μ; conidiis ellipticis vel paene globosis, 2.8–3.4μ diam., pallido flavo-viridibus lævibusque, coloniis gelatinam cito liquefacientibus, alkalinis lacmo.

Habitat, in fungis putrescentibus, Boletis, Polyporis; Storrs, Conn.; Paris, Gallia.

Cultivated in gelatin or potato agar, green or yellowish green, becoming gray-green or gray when old (remaining green in sugar media), floccose, spreading more rapidly in young cultures by aerial stolons than by submerged hyphæ (i. e., the submerged mycelium seems to arise from the aerial rather than vice versa); reverse of colony not colored or partly yellow; conidiophores arising as short branches (100μ or less in length) from aerial hyphæ, or arising separately 300μ or more in length especially at the margins of older colonies; conidial fructification 40–80 more rarely up to 170μ in length, composed of short appressed branches and numerous conidiiferous cells densely crowded at the base bearing very loosely divergent chains of conidia; sometimes the lowest branch diverges so that the fructification appears double; conidiiferous cells 10 by 3μ; conidia slightly elliptical or globose, 2.8–3.4μ, smooth, yellowish green in mass, almost hyaline by transmitted light; colonies liquefy gelatin very rapidly, with a strong alkaline reaction to litmus.

Habitat, decaying fungi, Boleti, Polypori; cultures from milk and ensilage. Collected repeatedly at Storrs, Conn.; once upon decaying *Boletus scaber* at the Jardin des Plantes in Paris, hence probably widely distributed. The stolon-producing character is so easily seen and so characteristic of this species as to seem adequate to distinguish it from all other species studied. This has been observed upon a decaying *Boletus* with a hand lens.

CULTURAL DATA.

Color white to yellowish green, deep green becoming yellowish brown or gray in old cultures; reverse, not colored (or slightly yellow); color in media, none or slight.

Odor, none.

Fifteen per cent gelatin in water, good growth, yellowish green; liquefaction, rapid in all gelatin media; litmus reaction, strongly alkaline. Potato agar, good growth, pale green to gray. Bean agar, good growth, pale green to gray. Potato plugs, good growth, deep green, transpired drops brown. Raulin's fluid, slow but characteristic colonies. Cohn's solution, typical growth.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, good growth up to 50 per cent, conidial areas persistently green (viridis to atro-viridis), acid reaction. Lactose 3 per cent, slow abnormal colonies, weak. Lactic acid 0.9 per cent, small colonies becoming alkaline. Levulose 3 per cent, typical colonies, alkaline reaction. Galactose 3 per cent, typical, alkaline reaction. Glycerin 3 per cent, good growth, not heavy. Potato starch 3 per cent, typical, drops yellow, fluid colorless.

Milk, typical colonies; curdling (0.25 per cent calcium chlorid added) in 1 week; digestion, rapid; color in milk, little or none.

At 37° C., killed; check at 20° C., good colony.

PENICILLIUM FUNICULOSUM n. sp.

Latin diagnosis.—Coloniis in gelatina vel agaro Solani tuberosi aut phaseoli cultis, atro-viridibus, late crescentibus, floccosis; parte aeria ex hyphis decumbentibus, ramosis, cæspitosis, late intricatis, et fasciculatis, conidiophoros breves gerentibus interdum hyphos secundarias albas floccosas lente evolvente; reverso rubescente demun atro-vinoso; substrato-aut lacte-aut gelatina, vinoso; conidiophoris (sine ramis) 20–80 usque 100μ longis, plerumque ex hyphis repentibus vel fasciculatis, interdum singulatim orientibus, fructibus conidicis usque 125–160μ longis, cum 1, 2 ramis alternatis, dein ramulis verticillatis, basidia in verticillos densos catenis conidiorum parallelis gerentibus; basidiis 10–14$\times$2–3μ, parallelis in verticillo, acuminatis; conidiis primum cylindricis, demum fusiformibusv el ellipticis, 3–4$\times$2–3μ, viridibus; conidiorum catenis solventibus submersis; coloniis gelatinam non liquefacientibus, acidis lacmo, siccantibus senescentibusque interdum coremiis paucis evolventibus.

In cultura, Storrs, Conn., 1905; communicavit Dr. E. A. Bessey, Miami, Fla., 1908.

Cultivated in gelatin or potato or bean agar, deep green, broadly spreading, surface closely floccose with procumbent hyphæ, tufts and ropes of hyphæ bearing lateral conidiophores; reverse becoming red, purple, or very dark purple, almost black, with the whole mass of medium colored; conidiphores short, 20–80 or 100μ, mostly perpendicular branches from trailing hyphæ, sometimes arising separately from the substratum; conidial fructification up to 125 or 160μ in length, with 1 or 2 alternate appressed branches bearing verticillate branchlets and dense verticils of parallel conidiiferous cells 10–14 by 2–3μ; conidia at first cylindrical, then elliptical or fusiform, 3–4 by 2–3μ, green, in chains which break up completely in fluid mounts; colonies not liquefying gelatin in 2 weeks, with acid reaction to litmus.

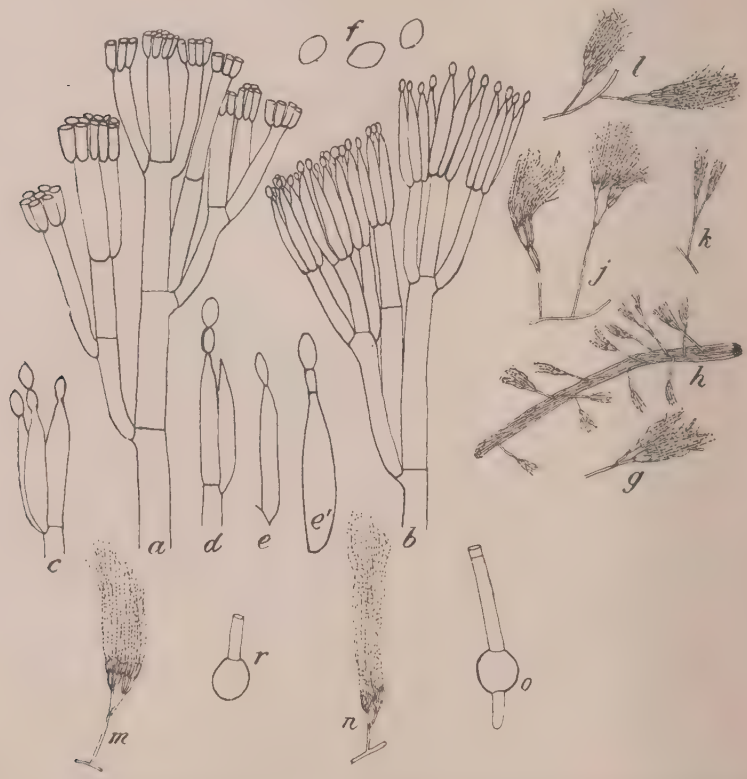

FIG. 27.—*Penicillium funiculosum: a, b, c, d, e, f*, conidial fructifications with conidiiferous cells and conidia (× 900, except *e*, 1,600); *g, h, j, k, l, m, n*, sketches of fructifications, separate and borne upon hyphæ and ropes of hyphæ (× 140). *o, r*, germination of conidia (× 900).

Found in accidental culture, Storrs, Conn., 1905; also received from Dr. E. A. Bessey, Miami, Fla., 1908. Easily recognized in culture.

CULTURAL DATA.

Color, deep green with secondary floccose masses of mycelium in some cultures; reverse and color in media, red to very dark red, or, colorless in certain media.

Odor, none.

Fifteen per cent gelatin in water, thin, widespread but characteristic growth; liquefaction, none or very slight; litmus reaction acid. Potato agar and bean agar, typical

colonies with red color in medium. Potato plugs, typical, reverse of colony and potato both deep red. Raulin's fluid, good growth, but no color in fluid. Cohn's solution, germination only.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar 3 per cent, good growth, but no red color. Lactose 3 per cent, very little growth. Lactic acid 0.9 per cent, little growth. Levulose 3 per cent, little growth. Galactose 3 per cent, little growth. Glycerin 3 per cent, germinated only, grew when sugar was added. Potato starch 3 per cent, good colonies, but no red color. Butteriat, rather small colonies with many delicate coremia, reverse of colonies red, with no color in fluid.

Milk, good growth with scattered coremia in old cultures; curdling (0.25 per cent calcium chlorid added) very slow—about 4 weeks; digestion slow or slight, no clear fluid; color in milk, colony deep red below, milk deep red (vinosus) at top, shading to white below, very slowly colored.

Grew about equally well at 37° C. and 20° C.

PENICILLIUM DECUMBENS n. sp.

Latin diagnosis.—Coloniis in gelatina pura vel agaro Solani tuberosi aut phaseoli cultis, griseo-glaucis, griseis, demun brunneolis, sparsis; in saccharo officinaro commixto densior, glaucescentibus; parte aeria ex hyphis decumbentibus vel stoloniformibus conidiophoros brevissimos gerentibus, denum cæspitulis albis densis hypharum sterilium secundariarum, conspersis; reverso incolorato; conidiophoris 20–100×3μ, basidüs 7–9×2–3μ, in uno verticillo denso gerentibus; fructibus conidicis ex catenis conidiorum primum in columno usque 100μ longo, mox, in capitulo conglutinato solutis; conidiis globosis, 2.5–3μ, vacuolatis, lævibus, primum pallide glaucis demum brunneolis; coloniis gelatinam non liquefa cientibus, alkalinis lacmo, saccharophilis, odorem in saccharo evolventibus.

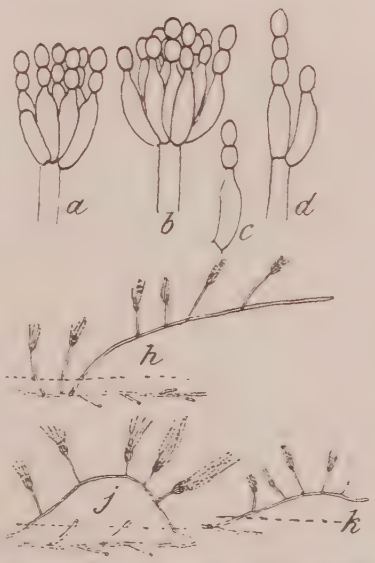

FIG. 28.—*Penicillium decumbens: a, b, c, d*, conidial fructification, a single verticil of conidiiferous cells (× 900); *h, j, k*, sketches of conidial fructifications, with diagram of habit and appearance of young culture on potato-agar (× 140).

Communicavit, Prof. P. H. Rolfs, Miami, Fla., 1905.

Cultivated in gelatin or potato agar, white to gray, gray-green ultimately yellowish brown, green in cultures with cane sugar, surface growth consisting of trailing or stolon-like hyphæ sparsely developed and so close to the substratum as to appear only as fertile hyphæ, bearing the conidiophores as short branches 20–100μ in length, in old colonies with dense tufts of sterile secondary mycelium scattered upon the surface; conidial fructifications consisting of single verticils of crowded conidiiferous cells, 7–9 by 2–3μ, bearing conidial chains first in loose columns up to 100μ in length but soon becoming enveloped and broken up in the drops of fluid secreted abundantly from the mycelium (*Gliocladium*-like; conidia globose 2.5–3μ, vacuolate, smooth, pale green then brownish in mass; colonies do not liquefy gelatin; give a weakly alkaline reaction to litmus; produce a definite odor in cultures containing cane sugar.

Contributed by Prof. P. H. Rolfs from Miami, Fla., 1905.

CULTURAL DATA.

Color gray, gray-green, often gray or gray-brown when old; reverse white; color in media, none.

Odor, distinct in cane-sugar media.

Fifteen per cent gelatin in water, medium growth, gray-green to brown when old; liquefaction, none; litmus reaction neutral. Potato agar and bean agar, rather small colonies, weak growth, grayish green to yellow-brown. Potato plugs, white to yellowish brown colonies, very weak growth. Raulin's fluid, rich growth, bright green, distinct odor. Cohn's solution, germination only.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, rich growth up to 30 per cent. Lactose 3 per cent, slight growth. Lactic acid 0.9 per cent, medium colony, light green. Levulose 3 per cent, small greenish colonies. Galactose 3 per cent, growth, faintly alkaline reaction. Glycerin 3 per cent, germination only. Potato starch 3 per cent, very slight growth. Butterfat, weak colonies.

Milk, not adapted to this species, colonies grow very slowly; curdling (0.25 per cent calcium chlorid added) slow—about 4 weeks; digestion, little or none; color in milk, none.

At 37° C. some growth; check at 20° C. better than 37° C.

PENICILLIUM DIVARICATUM n. sp.

Latin diagnosis.—Coloniis in gelatina vel agaro phaseoli cultis, avellaneis, nunquam viridibus, in substrato late crescentibus; parte aria ex hyphis fertilibus intricatis, demum fere pulverulenta; reverso incolorato; hyphis fertilibus septatis, plerumque brevibus, repentibus vel adscendentibus; fructibus conidicis aut terminalibus aut lateralibus ex hyphis fertilibus repentibus ex verticillis sessiles ramorum et basidiorum, irregulariter in hyphis fertilibus orientibus; basidiis 15–20×3μ, sterigmatibus longis acuminatis, in baside confertis, apice late divergentibus, catenas longas conidiorum gerentibus; conidiis ellipticis vel fusiformibus, 5–7×2.5–3μ, avellaneis, 10μ incrassatis 2–3 tubis germinantibus; coloniis gelatinam non liquefacientibus, alkalinis lacmo.

Legit, C. Thom, Storrs, Conn.

Cultivated in gelatin or bean agar, yellowish brown (avellaneous), never green, broadly spreading in the substratum; superficial growth consisting only of closely woven fertile hyphæ, becoming powdery in appearance when mature; reverse of colony not discolored; fertile hyphæ septate, usually short, mostly creeping; conidial fructifications either terminal or on short branches of creeping or partially erect hyphæ, consisting of separate conidiiferous cells, of verticils, or of series of verticils of branchlets and conidiiferous cells irregularly distributed along the fertile hyphæ; conidiiferous cells 15–20 by 3μ, with long acuminate sterigmata, broadly divergent at the apices and bearing long chains of conidia; conidia elliptical or fusiform, 5–7 by 2.5–3μ, yellowish to brownish, swelling in germination to 10μ and producing 2 or more tubes; does not liquefy gelatin; litmus reaction alkaline.

Unmistakable when once seen in culture. Found in a mucilage bottle, Storrs, Conn., 1904. Later contributed by Prof. G. F. Atkinson from North Carolina.

In common with several other forms included in the genus *Penicillium*, the conidial fructifications of this species are not strictly penicillate and terminal. Every gradation is found from fruiting systems typical of the genus to simple chains of conidia borne by single cells or basidia upon prostrate or even submerged hyphæ. It partakes, however, of the cultural character of the species of the genus, as shown by its copious growth upon many different substrata.

CULTURAL DATA.

Color light clay to chocolate or yellow (avellaneous, nearly) to darker, approaching brownish yellow, never green; reverse uncolored; color in media, none.

Odor, none.

Fifteen per cent gelatin in water, good growth; liquefaction, none; litmus reaction, acid. Potato agar, bean agar, and potato plugs, typical. Raulin's fluid, typical. Cohn's solution, germination only.

Synthetic fluid (Dox's) carbon supplied as: Cane sugar, good growth up to 30 per cent, acid reaction. Lactose 3 per cent, slight growth. Lactic acid 0.9 per cent, medium growth, not fully normal. Levulose 3 per cent, small development. Galac-

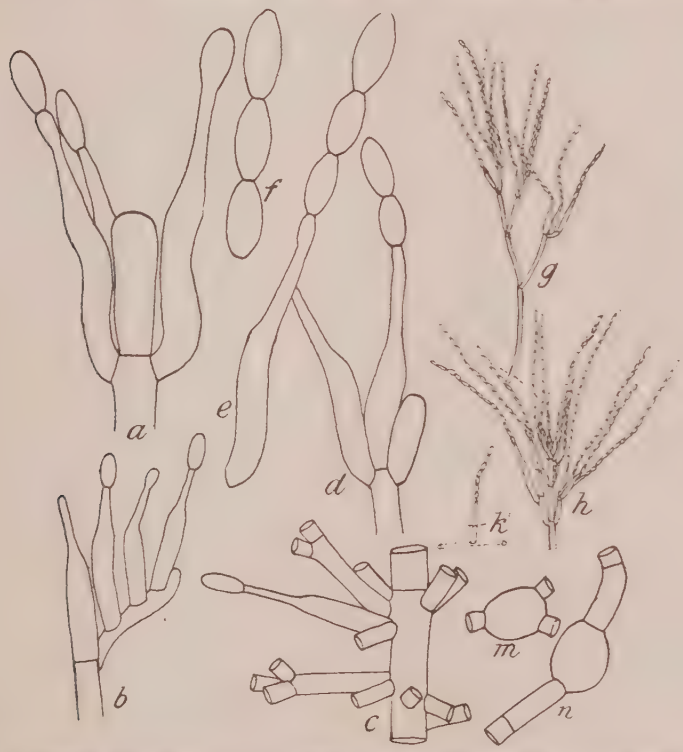

FIG. 29.—*Penicillium divaricatum: a, d, e, f.* conidiiferous cells, conidia and their arrangement (× 1,600); *b, c,* irregular types of arrangement; *g, h, k,* sketches of conidial fructification (× 200); *m, n,* germination of conidia (× 900).

tose 3 per cent, slow growth, reaction neutral. Glycerin 3 per cent, germinated only; grew when sugar was added. Potato starch 3 per cent, typical. Butter fat, weak growth, but characteristic fruiting.

Milk, slow and weak colonies; curdling (0.25 per cent calcium chlorid added) slow—14 days or more; digestion, slow and slight; color in milk, none.

Colonies grew better at 37° C. than at 20° C.

PENICILLIUM LILACINUM n. sp.

Latin diagnosis.—Coloniis in gelatina vel agaro phaseoli cultis, albis, vel albis demum pallide lilacinis imprimis in saccharo officinaro commixto, floccosis; hyphis aereis ramosis, adscendentibus, septatis, 3μ cr., ramos fertiles brevissimis gerentibus; reverso

incolorato; fructibus conidicis usque 100 longis, e basidiis sessilibus, solitariis vel verticillatis, aut, e ramis brevissimis vel apicibus hyphorum aeriorum, 1, 2, 3 verticillos ramulorum et basidiorum, catenas longas et divergentes conidiorum gerentum; basidiis basidibus incrassatis, apicibus acuminatis et divergentibus, 7–10 longis; conidiis 2.5–3×2μ ellipticis, lævibus, pallide lilacinis.

Coloniis gelatinam lente liquefacientibus, alkalinis lacmo.

Comm., Prof. G. F. Atkinson et C. W. Edgarton, Ithaca, N. Y.

Cultivated in pure gelatin or bean agar white, white to pale lilac in cultures containing sugars, more or less loosely floccose with hyphæ branched, septate, ascending, 3μ in diameter, producing conidial masses upon very short branches irregularly distributed, or becoming conidiophores toward the apex; reverse of colony not discolored; conidial fructifications up to 100μ in length, consisting of solitary, sessile conidiiferous cells, or verticils of conidiiferous cells, or short branches bearing 1, 2, or 3 verticils of branchlets and conidiiferous cells with long, tangled chains of conidia. Conidiiferous cells flask-shaped, divergent at the apices, acuminate, 7–10μ in length; conidia elliptical, smooth, 2. 5–3 by 2μ, thin walled, pale lilac. Colonies slowly liquefy gelatin, with strongly alkaline reaction.

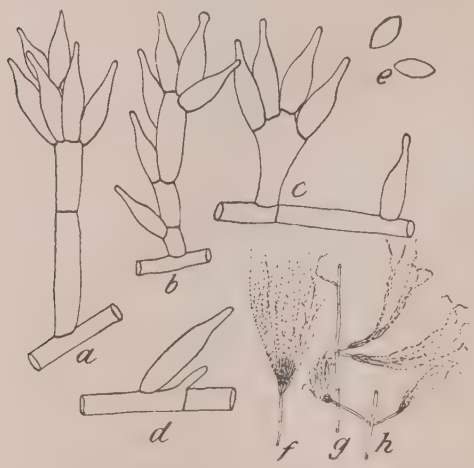

FIG. 30.—*Penicillium lilacinum:* *a, b, c,* short conidiophores and verticils of conidiiferous cells showing the various branching and arrangement of cells (× 1,600); *d*, conidiiferous cell, solitary and sessile on an aerial hypha, not uncommon in this species (× 1,600); *e*, conidia (× 1,600); *f, g, h*, sketches of conidial fructifications, varying from a single chain to a typical penicillate form (× 260).

Received from Prof. G. F. Atkinson and C. W. Edgarton, Ithaca, N. Y.

A relationship of this species to the common green forms is very doubtful. The chains of conidia produced break up so quickly and completely in mounting in fluid for examination that it is often difficult to find even a single conidium attached to its sterigma. The hyphæ with branches and basidial cells, aside from the production of long conidial chains, might readily be placed in any one of several hyphomycete genera. The form of conidial fructification varies from a single conidiiferous cell or basidium with a chain of conidia upon an aerial hypha to a single verticil, or a branch with two or three successive verticils and even to a terminal fructification allying it with the typical penicillate forms.

CULTURAL DATA.

Color white to a characteristic lilac shade; reverse of colony white; color in media, none.

Odor, none.

Fifteen per cent gelatin in water, fair growth, not heavy, white; liquefaction rather slow—14–16 days, litmus reaction strongly blue. Peptone milk sugar gela-

tin (Conn's), liquefied in 2 weeks, white colonies. Potato plugs, white colony; Cohn's solution, very weak growth but typical lilac color.

Synthetic fluid (Dox's), carbon applied as: Cane sugar 3 per cent to 30 per cent, good growth with typical lilac color, alkaline reaction, no fermentation. Lactose 3 per cent, germination only, slight growth. Lactic acid 0.9 per cent, germination only. Levulose 3 per cent, slow growth. Galactose 3 per cent, slow development with alkaline reaction. Glycerin 3 per cent, not characteristic, little more than germination. Butterfat, typical colony giving brownish color to fluid and causing drops of yellow oil to separate out.

Milk, curdling, slow; digestion, slow; color in milk, none.

At 37° C., best growth; at 20° C., good growth.

PENICILLIUM INTRICATUM n. sp.

Latin diagnosis.—Coloniis in gelatina vel agaro phaseoli cultis, albis, griseis, griseo-glaucis, demum griseis, lente fere fuligineis, floccosis; zonatis; parte aeria usque 1–3 mm. cr., ex hyphis aereis ramosis dense intricatis; reverso incolorato vel sulphureo interdum lente avellaneo; substrato sulphureo colorato; conidiophoris interdum terminalibus plerumque ex hyphis aereis brevibus 30–50μ ramosis; fructibus conidicis 50–100μ usque 140μ longis—multo longioribus in substratis saccharinis—ex verticillo basidiorum, vel ex 1–3 verticillis basidiorum in ramis divergentibus, vel ex verticillis ramulorum et basidiorum eodem verticillo, catenis conidiorum saepe columno laxe convergentibus; basidiis 8–10×2–2.5μ, paucis (4–10) verticillo, cum catenis basidiorum divergentibus; conidiis ellipticis vel globosis, hyalinis, vel pallide glaucis, 2.5–3μ diam., lævibus, leptodermibus, intus granulosis, in catenis manentibus submersis; coloniis gelatinam nonliquefacientibus, alkalinis lacmo.

FIG. 31.—*Penicillium intricatum: a, b, c,* conidial fructification, conidiiferous cell, conidial chain (× 1,000); *d, e, f,* sketches of conidiophores, branching and arrangement (× 260).

Culturæ ex humo, Prof. W. M. Esten, Storrs, Conn., 1907.

Colonies upon gelatin or bean agar, white, gray, greenish gray, when old gray or smoky, floccose, becoming a mass of interwoven hyphæ and ropes of hyphæ 1–3 mm. in thickness; reverse of colony and substratum not colored in bean agar, more or less sulphur yellow or even brownish in sugar media; conidiophores sometimes terminal, more commonly branches of aerial hyphæ 30–50μ in length; conidial fructifications 50–100 up to 140μ in length, or much longer in old sugar cultures, consisting of simple verticils of conidiiferous cells, or of 1–3 verticils upon divergent branchlets, or of branchlets and conidiiferous cells in the same verticil; conidiiferous cells 8–10 by 2–2.5μ, few (4 to 10) in each verticil, bearing more or less divergent chains of conidia frequently aggregated into a loose column; conidia elliptical or globose, hyaline or pale greenish, 2.5–3μ diameter, smooth, thin walled, granular within, remaining in chains in fluid mounts; colonies alkaline to litmus, not liquefying gelatin.

Found in cultures from soil, Storrs, Conn., by Prof. W. M. Esten, 1907.

CULTURAL DATA.

Color white or greenish gray not green, grayish to brown or drab; reverse and medium uncolored or sulphur-yellow in some media; litmus reaction strongly alkaline. Potato and bean agar, typical. Potato plugs, weak growth, not adapted to this species. Cohn's solution, weak growth, yellowish-green colonies.

Synthetic fluid (Dox's) carbon supplied as: Cane sugar, grew well up to 30 per cent. Lactose 10 per cent, good colonies. Levulose 3 per cent, good growth, yellowish mycelium, fluid yellowish, alkaline. Galactose 3 per cent, typical alkaline reaction. Glycerin 3 per cent, slow growth. Butterfat, good growth, reverse yellow, fat little changed.

Milk, fruiting areas upon glass, mycelium in contact with milk sulphur-yellow; curdling (0.25 per cent calcium chlorid added) very slow—3 weeks; digestion very slow in normal or acid milk, rapid when alkaline; color in milk, none.

At 37° C. grew more rapidly than at 20° C.

PENICILLIUM SPINULOSUM n. sp.

Latin diagnosis.- Coloniis in gelatina vel agaro phaseoli cultis, atro-viridibus, demum fere atris, cito et late in substrato crescentibus, margine sterili lata juvenilibus; parte aeria ex conidiophoris et ex hyphis floccosis sparsis composita; reverso incolorato; conidiophoris 105–300×3–3.5μ, vel longioribus, apice 5μ incrassato, verticillum basidiorum 9.5–11×2–3μ gerente; fructibus conidicis in columno denso 300 usque 500×15–30μ ex catenis conidiorum compositis; conidiis pyriformibus vel globosis, 3.2–3.5×3.6–4μ, leptodermibus, primum lævibus demum ministissime spinulosis; coloniis gelatinam lente liquefacientibus, acidis lacmo.

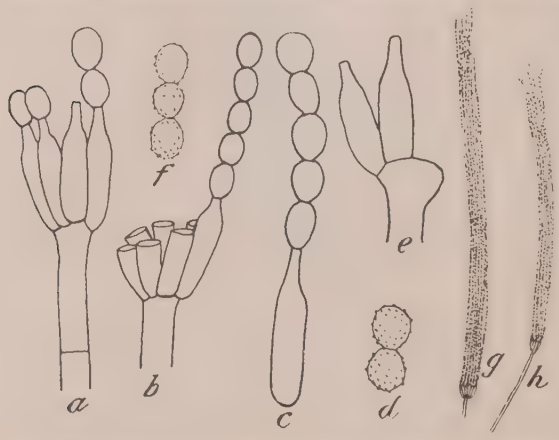

FIG. 32. *Penicillium spinulosum:* *a*, *b*, conidial fructifications consisting of single verticils of conidiiferous cells (× 900); *c*, conidiiferous cell with chain of young conidia smooth (× 900); *d*, *f*, ripe conidia, delicately echinulate (× 900); *e*, swollen end of conidiophore bearing conidiiferous cells (× 900); *g*, *h*, sketches of conidial fructifications (× 1,400).

In cultura in laboratorium, Hannover, Germania.

Cultivated upon gelatin or bean agar, deep green, spreading broadly in the substratum with broad sterile margin when young; aerial portion consisting of conidiophores and scattered aerial hyphæ; reverse of colony not discolored; conidiophores 150–300μ or longer by 3–3.5μ, with apex enlarged to 5μ in diameter, bearing a single verticil of conidiiferous cells 9.5–11 by 2–3μ; conidial fructification a close column of conidial chains up to 300 or even 500μ in length by 15–30μ; conidia pyriform to globose, 3.2–3.5 by 3.6–4μ, very thin walled, smooth at first then delicately spinulose or verrucose, yellowish green then almost smoky; liquefying gelatin slowly, with strongly acid reaction.

Found as a contamination of another species of *Penicillium* obtained in Doctor Wehmer's laboratory at Hanover, Germany. Easily recognized and cultivated.

CULTURAL DATA.

Color deep dull green; reverse cream, or slight traces of pink or violet; color in media, none.

Odor, none.

Fifteen per cent gelatin in water, slow but typical; liquefaction, rather slow and variable; litmus reaction, acid. Potato and bean agar and potato plugs, typical, producing a very heavy layer of dark-green conidia when cane sugar is added. Raulin's fluid, typical. Cohn's solution, germination only.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, grows well up to 50 per cent solution with acid reaction. Lactose 3 per cent, weak growth. Levulose 3 per cent, typical. Glycerin 3 per cent, half normal growth. Potato starch 3 per cent, typical. Butterfat, rich growth.

Milk, colonies grow very slowly; curdling (0.25 per cent calcium chlorid added) very slow; digestion, very slight; color, none.

At 37° C. grew better than check at 20° C.

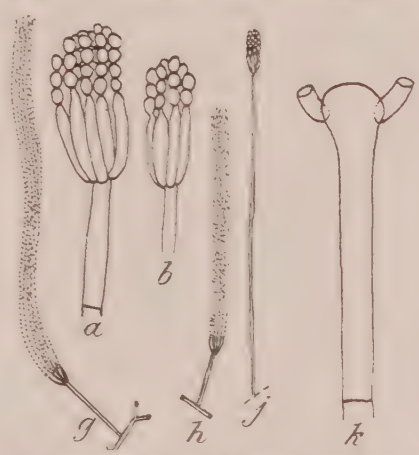

FIG. 38.—*Penicillium* No. 28: *a*, *b*, conidial fructifications each a single vertical × 90; *g*, *h*, *j* sketches of conidiophores and fructification × 140; *k*, tip of conidiophore, swollen at apex, bases of lowest two conidiiferous cells (× 1,600).

PENICILLIUM No. 28.

Colonies upon sugar gelatin and potato or bean agar, gray-green with broad white border when growing, floccose, tangled tufts of hyphæ and ropes of hyphæ spreading indeterminately upon the substratum, reverse yellow or tan on media containing sugar, conidiophores arising direct from the substratum as short lateral branches from 38–160μ in length, 3μ in diameter, swelling to 5μ at apex, from aerial hyphæ or ropes of hyphæ. Conidial fructification a simple column, 300μ or even 500μ in length by 10–15μ in diameter, produced from a single whorl of conidiiferous cells at the apex of the conidiophore. Conidia elliptical to globose, 2–3μ or 2–2.4μ by 3–3.3μ in diameter, light yellowish green in mass, smooth. Colonies liquefy sugar gelatin. Litmus reaction strongly acid.

Grows readily upon all common media.

Found at Storrs, Conn., upon decaying mushroom. Very characteristic and readily recognized from others with related morphology.

CULTURAL DATA.

Color gray-green; reverse yellow, or tan when sugar is present; color in media, more or less yellow, according to media.

Fifteen per cent gelatin in water, good growth, clear green; liquefaction slow—2 weeks or more; litmus reaction acid. Potato agar, typical growth, acid reactions. Bean agar, typical growth, acid reactions. Raulin's fluid, good growth, edges pink, fluid brownish fluorescent. Cohn's solution, weak growth.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, good growth up to 50 per cent, acid reaction. Lactose 3 per cent, slow but fairly typical growth. Lactic

acid 0.9 per cent, fair growth, not good. Levulose 3 per cent, good but slow colonies, acid reactions. Glycerin 3 per cent, medium growth, pink tinge to fluid. Potato starch 3 per cent, good growth, yellow drops of transpired fluid, fluid tinged yellow at top only. Butterfat, rich growth, fluid reddish brown.

Milk, good growth, acid in litmus milk; curdling (0.25 per cent calcium chlorid added) in 8–9 days, good curd; digestion slow, incomplete; color, pale yellow in digested fluid.

At 37° C., killed; check at 20° C., good.

SPECIES FORMING PINK SCLEROTIA.

Four races have been found in which pink sclerotia are regularly formed in culture. These sclerotia are elliptical to globose and from 200 to 500μ in diameter. They begin to be formed within the first week in richly nourished cultures. Although examined repeatedly, no trace of ascus formation has yet been found. These forms are included here under their serial numbers, 29, 30, 31, and 32, rather than with specific names. The descriptions and figures introduced will, it is thought, identify these organisms clearly in their penicillium form, but the uniformity of sclerotium production makes ascus production so probable under proper conditions that it seems best not to give specific names to this imperfect form when some of them may be already recognizable by others or by further investigation.

Penicillium No. 29.

Colonies grown upon gelatin and potato or bean agar white to gray-green, sometimes partly clear green, becoming zonate with rings of pink sclerotia in age, sparsely or loosely floccose, indeterminate broadly spreading margins persistently white, slightly yellow below. Conidiophores 80–200μ or even 400μ by 3–5μ commonly 150–200μ in length as branches, usually perpendicular, from hyphæ 4–5μ in diameter. Conidial fructification a single verticil of rather few (about 12–15) conidiiferous cells 9 by 2μ, producing chains of conidia in a loose column 150–250μ or even 400μ by 20–30μ. Conidia elliptical, 3–3.6 by 2.3–2.8μ, smooth, very pale blue (transmitted light). Sclerotia in loose networks of mycelium, numerous, pink, elliptical to globose, 150–300μ in diameter. These begin to appear in one week in gelatin cultures. Colonies liquefy sugar gelatin slowly but completely in 10–12 days. Give a strong acid reaction with litmus media.

Characterized by the production of large numbers of pink sclerotia with comparatively small quantities of conidia, whereas the next form (Penicillium No. 30) produces few sclerotia and great quantities of conidia.

Collected at Storrs, Conn., on decaying mushroom. Probably not closely related to the common species of *Penicillium*, but its occurrence in culture and ready adaptation to all media tried, in numerous cultures, justify its inclusion with these species.

CULTURAL DATA.

Color white to gray or green, with many pink sclerotia; reverse colorless or slightly salmon; color in media slightly yellow in some media.

Odor, none.

Fifteen per cent gelatin in water, good growth; liquefaction, 15 days; litmus reaction alkaline. Potato agar and bean agar, typical colonies, white or gray, with few green areas and abundant pink sclerotia. Raulin's fluid, typical. Colonies upon

media with cane sugar produce sclerotia more numerously and more quickly (5 days than without sugar. Butterfat as a source of carbon in Dox's fluid, typical colonies.

Milk, typical; curdling (0.25 per cent calcium chlorid added) in 9 days; digestion, slow; color in milk, none.

Penicillium No. 30.

Colonies on sugar gelatin and potato or bean agar gray-green or green persistently, surface growth mostly of crowded conidiophores and producing pink sclerotia at the surface or partly embedded in the substratum 150–300μ in diameter, broadly spreading. Conidiophores from 240–525μ, usually about 300μ, in length either arising separately from the substratum or as branches very close to its surface. Conidial fructification a single verticil of conidiiferous cells bearing conidia in a close column up to 500μ in length by 15–30μ. Conidia elliptical or subglobose 2.5 by 3μ or 3μ, with a slight greenish color. Colonies liquefy sugar gelatin rather slowly, and give an acid reaction with litmus.

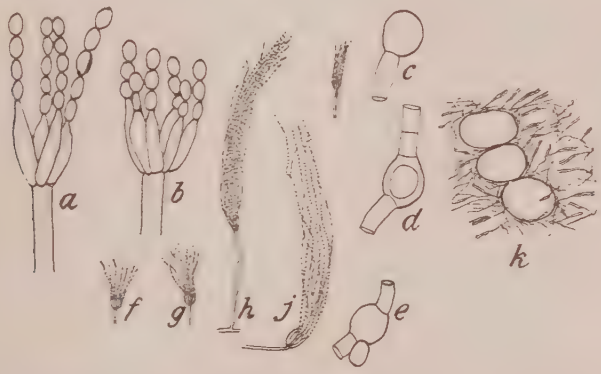

FIG. 34.—*Penicillium* No. 29: *a*, *b*, conidiophore and verticil of conidiiferous cells (× 900); *c*, *d*, *e*, germination of conidia (× 900); *f*, *g*, *h*, *j*, sketches of conidial fructifications (× 140); *k*, diagrammatic sketch from photomicrograph showing relations of sclerotia and conidial fructifications.

Apparently related to No. 29, but differing in the length and density of its column of conidia, in the position of the sclerotia, in habit, in culture, and in its acid reaction.

Collected at Storrs, Conn., upon decaying *Lactarius vellereus*, September, 1904.

CULTURAL DATA.

Color green or grayish green, persistently, with abundant pink sclerotia; reverse uncolored; color in media, none.

Odor, none.

Fifteen per cent gelatin in water, typical; liquefaction rather slow; litmus reaction acid. Potato agar and bean agar, typical. Potato plugs, typical. Cohn's solution, germination only.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, rich growth. Lactose 3 per cent, slowly typical colonies, acid reaction. Lactic acid 0.9 per cent, weak growth. Levulose 3 per cent, medium growth. Galactose 3 per cent, typical. Glycerin 3 per cent, very weak growth. Butterfat, slow, but characteristic colonies.

Milk, curdling (0.25 per cent calcium chlorid added) in 9 days; digestion, slow but complete; color in milk, none.

At 37° C., no growth; grew when cooled; check at 20° C., typical.

Penicillium No. 31.

Colonies upon gelatin and potato or bean agar from white to gray to gray-green mostly white, with few areas of green conidia sprinkled with pink sclerotia, sparsely floccose, broadly spreading. Conidiophores branching from aerial hyphæ, very short to 380μ in length, commonly 150–240μ, conidial fructification with a single verticil or once branched with branch, conidiiferous cells and chains of conidia divergent, up to 140μ in length, but usually much less. Conidia 2.5–3μ globose, smooth, rarely found in quantity to color the colony. Sclerotia elliptical or globose, 160–330μ, pink, developed in 10–15 days. No asci have been secured. Colonies liquefy sugar gelatin rapidly and give a stongly alkaline reaction to litmus in the same cultures.

Grows readily in conidial transfers upon all common media.

Collected upon decaying *Clavaria* at Storrs, Conn., September, 1904. Identical culture sent from Cambridge, Mass., by Dr. A. F. Blakeslee in culture obtained from fruit imported from Porto Rico.

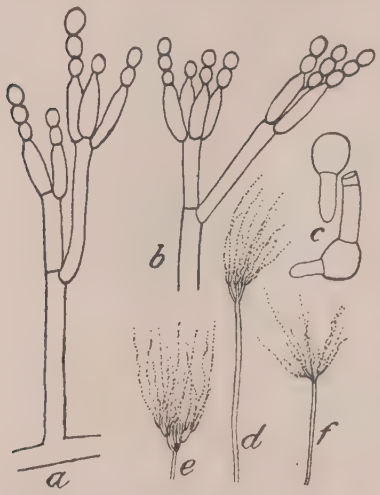

Fig. 35.—*Penicillium* No. 31: *a, b,* branching of conidiophore (× 900); *c,* germination of conidia (× 900); *d, e, f,* sketches of conidiophores.

CULTURAL DATA.

Color white or gray, conidial areas gray-green, very numerous pink sclerotia; reverse colorless or with yellow areas; color in media, none or slightly yellowish. Odor, none.

Fifteen per cent gelatin in water, typical white or gray colonies; liquefaction rapid; litmus reaction alkaline. Potato and bean agar, typical, cultures with sugar added become distinctly greener than others. Potato plugs, typical, white or gray with greenish areas, sclerotia, and crystal drops of transpired fluid. Raulin's fluid, some growth, not entirely typical. Cohn's solution, weak development but characteristic.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar 1.5–20 per cent, typical growth. Lactose 3 per cent, weak growth. Lactic acid 0.9 per cent, no growth. Levulose 3 per cent, typical. Galactose 3 per cent, typical, alkaline. Glycerin 3 per cent, slight growth. Butterfat, typical growth.

Milk, curdling (0.25 per cent calcium chlorid added) in 9 days; digestion complete; color, none.

At 37° C., no growth, grew when cooled; check at 20° C., typical.

Penicillium No. 32.

Colonies upon milk-sugar-gelatin and potato or bean agar gray-green; floccose, but with aerial part mostly long conidiophores and few vegetative hyphæ, slightly yellowish to pronounced salmon color below; broadly spreading; developing elliptical to globose sclerotia 150–200μ in diameter at the surface of the substratum in 2–3 weeks. Conidiophores 200–500μ by 3–4μ. Conidial fructification a verticil of 3–5 branches 10–17μ by 2–3μ rarely a secondary verticil, each bearing a dense verticil of conidiiferous cells, 8–10μ by 2μ producing long, parallel, or slightly divergent chains of conidia. Conidia elliptical or fusiform, 3.5–4μ by 2–3μ, green, granular within, smooth, swelling in germination to 6μ and producing from one to several germ tubes. Colonies slowly liquefy milk-sugar-gelatin and produce purple or neutral colors in litmus media.

Sent by Prof. P. H. Rolfs from Miami, Fla., upon portion of pineapple, March, 1905.

CULTURAL DATA.

Color gray-green, with scattered white to pink sclerotia; reverse, sulphur-yellowish to pronounced salmon; color in media reddish or yellowish in special cases, others none. Odor, none.

Fifteen per cent gelatin in water, typical; liquefaction, none in 15 days, later very slow liquefaction; litmus reaction neutral, leaves both acid and alkaline media purple-blue. Potato agar and bean agar, typical, slightly thinner than gelatin cultures, gray-green without sugar, clear green with cane sugar. Potato plugs, typical, transpires yellow drops which become very dark yellow (balsam). Raulin's fluid, good colonies becoming rosy below. Cohn's solution, small colonies, fluid slightly yellow.

Synthetic fluid (Dox's), carbon supplied as: Cane sugar, grew in solutions up to 30 per cent with acid reaction. Lactose 3 per cent, very slow growth of small characteristic colonies. Lactic acid 0.9 per cent, good growth, light green. Levulose 3 per

FIG. 36.—*Penicillium* No. 32: *a, b, d*, branching of conidial fructifications (*a* × 1,400, *b* and *d*, × 900); *c*, a single secondary verticil (×1,400); *g, h, j, k*, sketches of fructifications of various ages (× 140); *m, n, o*, germination of conidia (× 900).

cent, very slow-growing but heavy colonies. Galactose 3 per cent, typical. Glycerin, very small colonies. Butterfat, typical colonies.

Milk, curdling (0.25 per cent calcium chlorid added) very slow; digestion, very slow; color in milk, none.

At 37° C., grew more rapidly than check at 20° C.

COMPARATIVE CULTURAL DATA.

A summary of accessory cultural data has already been given for each species in connection with the descriptions. Many series of cultures have been made with numerous media to obtain data as to the ability of the species studied to grow upon particular media or under particular conditions. It has been possible thus to determine the relative activity of single species and groups of species. Although particular species in these cultures have shown unique differences which assist in their differentiation, the most valuable

result of comparative culture is found in the separation of the series into groups of races or species which resemble each other closely in their metabolic activities. Part of these experiments are tabulated in Tables 2, 3, 4, 5, and 6, and will be discussed in the following sections.

Since the complex composition of the media in common use for cultural work makes the analysis of the data obtained impossible, it was first necessary to determine the reactions of these species to some of the individual substances of which these media are composed. It has already been noted that gelatin alone in distilled water sustains growth in the large majority of the species of this genus. These cultures in certain species lack green color, which, however, becomes present on using the peptones and sugars added to gelatin in most formulæ. Such media are still too complex to make close analysis of results possible.[a]

Many of the determinations given were made in duplicate and in some cases the entire series was repeated one to several times. In inoculating cultures for this work conidia were transferred to the tubes in large numbers, so that their presence could be detected by examination with a lens. Where species failed to grow, the doubts of inoculation were commonly dispelled by the addition of cane sugar, which permitted the conidia to develop normally if present and still viable. The presence of germinated conidia upon the surface of a medium is good evidence of proper inoculation. The data given are believed, therefore, to represent with a fair degree of accuracy the comparative cultural reactions of the species used.

In reporting these series of comparative cultures, the data have been tabulated as far as possible for convenient comparison of the relative activity of the different forms. (See tables beginning on p. 98.) The names as far as determined are given, together with the cultural number, in Table 1. In the remaining tables the numbers are repeated without the names. In studying the tables a reference to cultural numbers will quickly locate the forms discussed.

CULTURES IN DISTILLED WATER.

To determine the possibilities of growth from food stored in the conidia, cultures were made in distilled water. Of forty-four strains under cultivation but six showed clearly descernible germination. None produced more than germ tubes hanging down into the fluid.

[a] This work was carried on in cooperation with Mr. A. W. Dox, who has studied the metabolism of the species concerned with cheese ripening (*P. camemberti* and *P. roqueforti*), as well as a few other species, under many conditions of culture, while the writer has conducted comparative studies of a large number of forms under more limited cultural conditions. All chemical questions arising throughout this work have been passed upon by Mr. Dox.

AGAR-AGAR AS A SOURCE OF FOOD.

Tubes of 1.5 per cent agar-agar in distilled water were inoculated with eighteen species of *Penicillium*. Sixteen of these produced growth. In all cases the colonies produced were very small, some of them barely discernible to the naked eye. Not one of them was distinctly colored by conidial masses, but in nearly every case some conidial fructifications were found. These cultures show that the species tested were able to obtain from the medium sufficient nourishment for very slight growth.

AGAR-AGAR AS A SOURCE OF CARBON.

Agar-agar is a carbohydrate and might serve as a source of carbon if other nutrients were supplied. One and one-half per cent of agar was therefore introduced into Mr. Dox's synthetic fluid, already containing all essential elements except carbon. Thirty-seven races of *Penicillium* were inoculated into this medium, and nearly every species produced some growth. Examined with the microscope, conidial fructifications were found in nearly every case, but in no case was the colony large enough or definite enough to affect the observer's estimate of results if such growth were added to or subtracted from the colonies upon nutrients really adapted to sustain the species studied. Dox's stock solution, with or without the addition of agar, was in this way shown to be a safe medium for the study of the metabolic reactions of these species to changed sources of carbon.

The possibility of error in the introduction of agar was shown in the following manner: One and one-half per cent of agar was introduced into Dox's fluid and 1.1 normal lactic acid added in quantity to make the whole 0.5 per cent acid. The medium was then autoclaved. After this treatment the agar refused to solidify. Tubes of this fluid were inoculated with nine different species of *Penicillium*. All except *P. brevicaule* grew well and produced colonies recognizable by their cultural characters. In introducing agar in such work it is therefore necessary to guard against the introduction of acid before dissolving the agar, since this changes the agar itself into other carbohydrates assimilable by fungi. Although parallel cultures were commonly made with agar, the studies of metabolism recorded in this paper were made in tube culture of the fluid nutrients only, to avoid possibilities of error.

VARIOUS SOURCES OF CARBON.

Cane sugar (*Tables 4 and 5*).—Cane sugar was added to Dox's fluid in the following percentages: 1.5, 3, 10, 20, 60, and 75. Of the species used, but one—*P. digitatum* of Saccardo—failed to grow typically. This, together with other work, indicates that this species is

incapable of assimilating nitrogen from the sodium nitrate of this solution. Four more forms—*P. brevicaule* and its closely related varieties, and *P. roseum* Link—reached normal appearance slowly. Other cultures indicate that these forms assimilate nitrogen in this form less readily than in organic combinations. This medium containing cane sugar in amounts as great as 20 per cent proved well adapted for all other species tried. The medium as used was neutral or slightly alkaline in reaction. Thirty-three of the forms inoculated into it produced pronounced acid reactions to litmus in a few days, i. e., were able to ferment this form of sugar. In prolonged culture with smaller amounts of sugar, some of these forms finally reduced the acidity and even brought about an alkaline reaction again; others remained acid as long as observed. Twelve forms failed to produce acidity. This number includes the four forms in which growth was delayed and *P. italicum*, *P. luteum*, *P. purpurogenum*, *P. roqueforti*, and *P. duclauxi*.

In cultures at a concentration of 60 per cent cane sugar, five forms produced typical colonies at once; six others slowly reached normal proportions; a few more grew fairly well; but fully half the species tried produced germination with but little further growth. Water in amount approximately to reduce the concentration to 35 per cent was added to the cultures that failed to produce normal colonies, and this was followed by the prompt recovery of several species which quickly reached normal development. Critical examination of the data obtained showed that closely related types responded in exactly the same manner to changed percentages of cane sugar. As a means of separating closely related forms, further determination seemed fruitless. Exact determination of the maximum percentages of cane sugar tolerated by particular species has not been completed. The inhibition is not a stoppage of all development at a definite critical concentration, but rather a gradual reduction of activities with the increasing concentration of the medium. Specific maxima and minima are therefore almost impossible to define. All determinations would therefore rest upon the judgment of the observer rather than upon fixed standards.

Lactose (*Tables 4 and 5*). Lactose was added to Dox's fluid in percentages up to 10 per cent. Prompt and normal development was determined in eight forms; nine more forms reached typical appearance more slowly. Only twelve of these produced definite acid reactions to litmus. Among the forms included in the seventeen, four groups of closely related organisms were found, namely, the *camemberti* group, Nos. 5, 6, 39; the common green group, Nos. 22, 23, 40; the *chrysogenum* group, Nos. 25, 26, 35, and 44; and the *brevicaule* group, Nos. 2, 3, and 4. With lactose as with cane sugar closely related forms give

approximately the same reaction in most cases. Three of these groups show acid and the fourth alkaline tests with litmus. A few of the remaining species continued to grow until after several weeks they reached almost normal development. In such cases the assimilation of carbon from lactose seems to be very slowly and with difficulty accomplished by a large proportion of the species studied, and to be practically impossible to some species.

Comparison of the litmus reactions produced by the various species with cane sugar and with lactose accounts for striking differences in this reaction when litmus is introduced into gelatin or agar media containing these two forms of sugar. A species which will ferment cane sugar and not ferment lactose will produce an acid reaction with one and an alkaline reaction in media containing the other.

Lactic acid (*Tables 4 and 5*).—Tubes were prepared containing Dox's fluid to which 0.9 per cent of lactic acid was added. Thirty forms were inoculated into this medium. Of these forms nine produced normal and typical colonies, showing but slight inhibiting effect from the acid. As many more cultures slowly became typical colonies. Nearly every form germinated and produced slight growth. In the *camemberti* group (Nos. 5, 6, 39) and some others the litmus reaction became alkaline. It was thus shown that a series of species could secure carbon from lactic acid and in doing so destroyed the acid character of the medium. The species which grew most rapidly in lactic acid were those which had developed best in the lactose solutions.

Levulose (*Tables 4 and 5*).—Tubes were prepared into which 2.5 per cent of levulose was introduced as the source of carbon. The results as tabulated may be seen to group together about the same species as the previous experiments, except that one or two forms were found to grow well in levulose that failed to grow with lactose or lactic acid as a source of carbon. Fourteen forms produced typical colonies without inhibiting effects, while five more slowly reached typical development.

Galactose (*Tables 4 and 5*).—A series of cultures was made in the same way with 3 per cent galactose as a source of carbon. Galactose proved much better adapted to supply carbon than either lactose or levulose. Twenty-five forms produced typical growth, and others grew more or less readily. This form of sugar therefore proved of but small assistance in the separation of species.

Glycerin (*Tables 4 and 5*).—Cultures offering carbon in the form of 3 per cent glycerin produced much less growth than those containing sugars. Eleven forms eventually reached fairly typical growth; four only of these showed no restraining effect of the medium. Of these, three are probably closely related if not merely races of a single species—the one most common in general cultural work in this

region. Apparently glycerin presents a form of carbon much less available for assimilation by species of this genus than the sugars.

Butterfat (*Table 4*).—To test the ability of these fungi to assimilate fat, butter was melted, strained, filtered through filter paper, and added to Dox's fluid. Although not chemically pure, perhaps, it is believed that the amounts of other nutrients would be too small to affect results. Only one form (*P. digitatum*) failed to grow. *P. luteum* gave only slight growth. The majority of forms, although growing slowly, produced typical or fairly characteristic colonies. The masses of fat were visibly much changed, becoming incrusted with a white substance in most cultures. In a few cultures the action of fungus caused the separation of the various fats, so that drops of yellow oil separated out from the remaining nonliquid matter.

Potato starch.—In one series twenty-seven forms were cultivated in Dox's fluid, containing 3 per cent of potato starch. All of the common species of the genus were found to grow normally upon a medium containing starch as the source of carbon. The characters in this medium were approximately the same as in the stock agar or gelatin cultures. Two species (*P. decumbens* and *P. digitatum*). which failed to grow well have since been shown to depend upon the presence of cane sugar for vigorous growth and green color to their spores. Similarly the same species grown upon plugs of potato failed to produce strong colonies of pronounced green color.

Malic and succinic acids.—Series of cultures were made with 1 per cent malic acid and with 1 per cent succinic acid as sources of carbon. All species germinated, but no species reached fully typical development in either series. Some few species produced slowly colonies of half or more of the normal size with conidial masses of typical color. Many of the species grew sufficiently to produce a few conidial fructifications recognizable with the hand lens. These two series emphasize the observation already made that species inoculated into a medium ill adapted to their nourishment will nevertheless grow and produce small amounts of fruit under widely different conditions even where normal growth is impossible.

CULTURES IN RAULIN'S FLUID AND COHN'S SOLUTION.

The comparative data for cultures in Raulin's fluid and in Cohn's solution are shown in Tables 3 and 5. Raulin's fluid, as given by Smith, is a highly acid medium and has been found very well adapted for the growth of certain species. The solution is, however, too complex to make analysis of cultural results upon it readily possible. It contains carbon in three different forms—tartaric acid, potassium carbonate, and magnesium carbonate—and nitrogen in two forms

in ammonium nitrate. Aside from data as to growth or failure to grow, culture in such a medium is fruitless.

Cohn's solution is also an acid medium, but its acidity is due to the presence of monopotassium phosphate (KH_2PO_4). In a series of cultures with Dox's formula it was shown that the monopotassium phosphate had practically no different effect upon cultures of these species than the dipotassium phosphate (K_2HPO_4).

Very few of the species were found to grow typically upon Cohn's solution, however, although nearly all germinated. Comparative work with Dox's formula shows that but few of the species studied are capable of normal assimilation of carbon from forms of tartaric acid. Only three of the species experimented with failed to show distinct germination. One of these inoculated with *P. camemberti* var. *rogeri*, remained four months without exhibiting any germination of the conidia. The conidia were then transferred with a platinum needle to a petri dish of gelatin; under these conditions the same spores developed into typical colonies without showing any ill effects of four months' immersion in the fluid which they were unable to assimilate.

In solutions nontoxic in character many species exhibit definite selective preferences for nutrient elements in particular chemical combinations. This selective adaptability to particular forms of nutrients differs greatly for different groups of species. Some forms of wide distribution seem adapted to produce typical growth upon quite varied chemical solutions. Other species (e. g., the rots of citrus fruits), equally wide in their distribution, are closely dependent upon particular forms of food, whereas between these extremes are many forms exhibiting preferences as to nutrients yet capable of development, although more slowly, in media containing nutrients in forms assimilated with difficulty. These experiments have offered no tangible evidence of rapid adaptation to media found unadapted at first to development. Such power of gradual adaptation is not excluded, however, by these experiments.

COLOR IN CONIDIAL AREAS.

In dealing with all species it has been found by repeated experiment that the green color of the spores is dependent upon the proper assimilation of the carbon element. Many of these species produce a brighter green when cane sugar is present than with any other form tried. Grown upon gelatin, or upon potato agar or bean agar (free from sugar), several species produce green masses of conidia which rapidly become some shade of gray, brown, or almost black in media without sugar, but when cane sugar is present they are deep green and remain unchanged for much longer periods. Certain other species entirely lack green color except when sugar is present.

A color determination is therefore dependent for its value upon knowledge of the form of carbon presented by the medium. These same species grown with lactose as a source of carbon respond exactly as they do in potato or bean agar or in gelatin free from sugar. Another series of forms are little if at all changed in color by changing the form of carbon presented, provided only that they are able to grow readily in the medium as presented. It seems therefore certain that very many widely distributed species are capable of assimilating carbon in very widely different chemical forms and to produce in such cases normal and typical growth and colors, whereas other species are entirely dependent for normal growth and color upon the presence of particular chemical combinations or groups of combinations. Study of the forms so responding likewise shows that the ubiquitous species are capable of assimilating carbon in the most varied combinations, whereas the forms lacking this power are mostly less common and more specialized.

EFFECT OF CONCENTRATED MEDIA.

Similarly great differences in the mass of the growth produced are directly attributable to the presence of nutrients easily available or in greater concentration. The formulæ usually recommended contain the nutrient used, in extremely dilute proportions. The determinations already given with different percentages of cane sugar show that these species are able to assimilate sugar in widely different concentrations. With the majority of forms studied there seems to be no deleterious effect from increasing the concentration of the nutrients offered until the solution has attained an osmotic pressure sufficient to inhibit growth by plasmolyzing the cells, or until the reduced percentage of water gradually reduced the rate of fungous growth. In one experiment nine species were inoculated into a medium containing 5 per cent cane sugar, 10 per cent Witte's peptone, and 5 per cent Liebig's extract. All species grew luxuriously. The mass of mycelium produced was much in excess of the results with ordinary culture media. One form normally producing sclerotia produced a very rich growth of mycelium, but no sclerotia. In media of higher concentrations the amount of growth—the mass of mycelium and conidia—is, however, so greatly increased as to render the discrimination of the character of the species more difficult by mechanical interference due to the quantity of material. For purposes of study, therefore, the usual formulæ really produce the more satisfactory growth in nearly every species, although from the standpoint of fungus development such media must be recognized as far below the optimum concentration for the species of this genus.

It must further be noted that in cultures containing cane sugar many species continue to produce conidia for a much longer period

than in solutions lacking sugar or some nutrient equally assimilable. In these cultures the quantity of conidia produced increases enormously, often becoming a layer over the whole surface of the colony half a millimeter or more in thickness. The presence of such masses of spores greatly complicates the study of the structure of the colonies, but is especially characteristic of such species.

THE GROUPING OF SPECIES.

Analysis of the cultural tables presented in the light of many series of comparative cultures makes possible the grouping together of particular races or species which possess common cultural characters. It is comparatively a simple matter to single out first the unique forms—those which are never green, those which produce a particular form of sclerotia, those which regularly produce prominent coremia, or those which produce striking colors in the substrata, even those associated with particular substrata. There remain, however, the large number of green forms which lack these striking characters. This large group comprises probably most of the forms which have masqueraded under the name *P. glaucum*. Lines of differentiation among these forms are more or less obscure. Kept in continuous culture races are easily differentiated with the eye by shades of color or habit, but characters of easily recognizable diagnostic value in written descriptions are more difficult to find. Inspection of cultural data shows, however, that there is a well-marked group of these forms which are able to ferment lactose as well as cane sugar. These comprise the *camemberti* group (Nos. 5, 6, 39), the *chrysogenum* group (Nos. 25, 26, 35, 44), and what we may call the "commune" group (Nos. 22, 23, 40). Among those not causing an acid reaction in lactose cultures is a series of rapid liquefiers of gelatin which have many characters in common (Nos. 12, 15, 24, 37, 38). All of these forms show special adaptability to growth in cane-sugar media. No. 15, *P. citrinum*, produces brilliant lemon color, especially in sugar media; No. 38 is given as *P. atramentosum*, from its blackening of the substrata in sugar media and in milk; No. 37 by developing green color when sugar is present, which color is lacking or evanescent without sugar. These forms differ in their color reactions in the medium, in the size and shape of their conidia, in the length and origins of their conidiophores, and in the arrangement of the elements in the conidial fructifications.

Another marked habit difference which holds true throughout many series of cultures is the tendency of colonies of certain species to spread rapidly over the whole surface of the substratum, whereas others are quite restricted in their habit of growth. In the first the developing margin is almost uniformly broad and while growing white, e. g., *P. roqueforti*, *P. italicum*, *P. chrysogenum*, *P. divaricatum*,

P. spinulosum, *P. expansum*, *P. brevicaule*, and such floccose forms as *P. camemberti* and *P. biforme;* the species of restricted habit with narrow growing border are represented by *P. citrinum* and *P. atramentosum* and their allies. The designation "spreading," "restricted," "with broad margin," or "with narrow margin" is descriptive for colonies of such species.

ODORS.

Although many persons seem to detect the presence of mold by its odor, very many of the species give but little definite odor. A few forms, some of them (e. g., *P. biforme*) always, others upon special media, produce difinite odors by which they can be recognized or placed in particular groups. Grown upon gelatin media, *P. brevicaule* produces a strong ammoniacal odor. A piece of moistened litmus paper held over such a colony will promptly give the alkaline reaction. This organism is recorded as emitting arsin from cultures containing arsenic in any form and it is said to be a very delicate test for the presence of that element [a] (Gosio). The two forms here described as varieties of *P. brevicaule* give exactly the same odor. In nearly every medium used *P. expansum* (the apple rot) gives a strong odor, which suggests decaying fruit to some. Once well distinguished, the presence of this organism can be detected even as a contamination wherever it occurs, by the odor alone. Another given here as *P. atramentosum* produces a very characteristic odor while digesting milk, defined by one as the odor of rancid walnuts, to another it suggested mice. A series of forms when grown upon cane sugar produce a very characteristic odor—an ester, according to the chemists to whom it was submitted—recognizable to the sense of smell, but not definable. The olive-colored orange rot (*P. digitatum*) gives this most strongly, but it is also given by *P. italicum*, *P. decumbens*, and No. 13. The odor of *P. claviforme* is found under nearly all cultural conditions, and would readily identify it were its big coremia not already very distinctive. The common and undefinable green group contains a series of races or forms, many of which give what is popularly called the smell of mold. In others of this group this odor is scarcely distinguishable.

ANAEROBIC CULTURES (WITH CARBON DIOXID).

The possibility of some species developing under anaerobic conditions was tested as follows: Vials were prepared with Dox's fluid having 5 per cent cane sugar as a source of carbon. This had already been shown to be an excellent medium for the growth of nearly all the species used. These vials, containing all the species herein described, were packed in a crate or test-tube basket and put into a Novy jar. The jar was then given in charge of Mr. Dox. The air was exhausted

[a] Gosio. Azione di alcune muffe sui composti fissi d'arsenico. Rivista d'Igiene e Sanita Pubblica, Rome, 1892. See page 201.

as completely as possible. Carbon dioxid washed through water and through sulphuric acid was carefully introduced. The exhaustion was repeated and the gas introduced a second and a third time. The cultures were then permitted to stand one week and examined. A culture of *Oidium lactis* among the species of *Penicillium* was found to have grown some. No species of *Penicillium* had produced a colony. The carbon dioxid was then three times exhausted and replaced by air and the jar permitted to stand a second week. Normal colonies of every species were produced. Clearly, therefore, no one of the species of *Penicillium* under experiment was capable of growing in an atmosphere of carbon dioxid, and no species was killed by exposure to such atmosphere.

INCUBATION TESTS.

The importance of temperature in determining the distribution of fungi in nature, and in controlling their presence in the household, the dairy, and the storage room, made the determination of the limits of temperature for the growth of these species desirable. The following incubation experiments were therefore made. (See Table 6.)

1. Incubation at 20° C. This was repeated several times with fully checked records.

2. Incubation at 37° C. (range of variation 35° to 38° C.) for six days; cultures then examined, recorded, and the incubator cooled to 20° C. for the succeeding six days.

3. Use of the ice thermostat.[a] Four series of cultures were made in bean-agar with 5 per cent cane sugar—a medium adapted to produce typical colonies of all species in a minimum of time. The temperature of the incubator was recorded eight times a day at three-hour intervals. The range of temperatures in the compartments used and the average of fifty-five observations taken in the first seven days were as follows:

Compartment 1, average 1.05° C., range 0.5° to 2° C.
Compartment 2, average 4° C., range 3.2° to 6° C.
Compartment 3, average 7° C., range 6° to 10° C.
Compartment 5, average 8.7° C., range 7° to 10.5° C.

Observations upon the cultures were made by the writer at 3 and 7 days and repeated, by the kindness of Miss Lucia McCullough, of the Bureau of Plant Industry, at 15, 23, and 29 days. For convenience of comparison, the notes from all these observations have been reduced as fairly as possible to a decimal code, in which germination of conidia without further growth is given as 0.1 and typical colonies at 1.0; fractions from 0.1 to 0.7 represent vegetative mycelium without colored conidial areas, and from 0.7 to 1.0 the

[a] By the courtesy of Dr. Erwin F. Smith the ice thermostat of the Bureau of Plant Industry of the United States Department of Agriculture was placed at my disposal. It was iced and regulated under his instructions.

completion of the typical colony. The figures are brought together in Table 6.

Data at 20° C.—The data at 20° C., as given in the first column of Table 6, are regarded as typical for the species studied and given as 1.0. Numerous series of cultures with all these forms under close observation in the incubator at 20° C. and in the laboratory where the temperatures used ranged from 15° to 25° C. or slightly higher have given approximately the same results. Within these limits, rise or fall in temperature affects the amount of growth or the stage of development of the colonies within a specified time without affecting the character of such growth. The differences between cultures grown at different temperatures within these general limits are quantitative, not qualitative. Unless made for a specific purpose cultures of these fungi may be safely grown outside the incubator without affecting their character, since the conditions in the ordinary working room are approximately those furnished to these forms by nature.

Data at 37° C.—At 37° C. thirteen forms showed normal development. Of these seven grew better at 37° C. than at 20° C., this number including but one well-known species—*P. luteum*. At the same temperature the spores of seven species were killed, including among these *P. italicum* and *P. digitatum*, the species destructive of citrus fruits. Of the green forms abundantly found, only one grew well at 37° C.—*P. chrysogenum*. The numerous green forms studied were not killed, but simply prevented from growing by the heat. Every form except those noted as killed developed normally in the same tubes as soon as cooled to 20° C.

Ice thermostat.—In compartment 1 of the ice thermostat, ranging from 0.5° to 2° C., 20 of the forms experimented with either produced germ tubes only or failed even to germinate in twenty-nine days. Of the remaining 18, only 6 produced colored conidial areas in that time. Several other species produced considerable masses of white mycelium.

In compartment 2, ranging from 3.2° to 6° C., with an average slightly above 4° C., 16 or 17 still showed germination only or complete inhibition; 11 showed colored conidia; several additional forms had germinated or produced distinguishable mycelium.

In compartment 3, with an average temperature about 7° C. and a range from 6° to 10° C., 16 forms produced colored fruit. Of these, 9 had reached the typical appearance of mature colonies of the species within the 29 days. Nine only remained without showing some mycelial growth in addition to germination.

In compartment 5, averaging about 9° C. and ranging from 7° to 10.5° C., all species had germinated and all but one had produced mycelium; 25 forms had developed colored conidia; 17 had produced colonies of typical appearance.

Study of the figures given shows a progressive increase in growth from the coldest to the warmest compartment. Examination of the cultures showed that at the lower limits of growth very many species produce colored fruit very slowly at temperatures at which vegetative mycelium is still developed quite rapidly. There often results, therefore, in cold temperatures a disproportionate growth of white mycelium and a tardy development of colored conidia, which often affects the appearance of the resulting colony considerably. Cultures grown under such conditions would be difficult to identify in many cases. The forms which grew most rapidly at 37° C. either failed to grow at the colder temperatures or responded very slowly. The large majority of the species, and especially those most commonly found in food materials, are seen to begin fairly rapid growth at temperatures within a few degrees of the freezing point. When taken from the ice thermostat all cultures which had failed to produce normal colonies grew quickly to typical appearance. There was, therefore, no injury attributable to continuation for 29 days at low temperature. The general effect of low temperatures upon these species is the suspension of or the reduction of the rate of development. In many cultures the beginnings of growth were difficult to detect and in most cases an exactly critical temperature is not determinable, since cultures not showing any growth in one week seem gradually to adjust themselves to conditions and produce mycelium in the succeeding weeks.

Eustace[6] has recorded that one of these species (*P. expansum* Link of this paper) will produce rot in storage apples where the temperature of the room as recorded does not rise above 32° F. (0° C.). When the time was extended to two months Petri-dish cultures under the same conditions produced small colonies. The experiments here recorded tend to suggest that very little growth will occur in most species at temperatures nearer than 2° C. to the freezing point, although many of them will germinate. Unpublished records of the temperatures of apples in storage, furnished by Mr. C. D. Jarvis, of this station, showed that the flesh of apples in storage was constantly from 1 to 2 degrees at least above that of the room. Allowing for the conductivity of the thermometer itself, the difference is probably somewhat greater. Both series of data indicate, however, that storage temperature to exclude fungous growth must be close to the freezing point. It is clear that low temperatures (above freezing) merely restrain growth, not entirely prevent it. It is also clear that by restraining the production of colored fruit many colonies would be rendered inconspicuous (although widely growing), thus accounting for the complaint so often heard with reference to dairy products taken from the refrigerators, that they turn green with mold very quickly.

SUMMARY OF DATA FROM COMPARATIVE CULTURE.

1. Species closely related in morphology and general appearance give closely similar reactions in culture under most conditions, but commonly show a few well-marked differences in special media or under special conditions.

2. Certain species will grow in media of widely differing composition; others require particular media for normal development.

3. Cane sugar in low concentrations is readily assimilated by every species studied.

4. Butterfat was attacked by nearly every species.

5. Lactose, galactose, levulose, and glycerin are assimilated by some species and not by others.

6. Potato starch produced normal growth in most species.

7. Very few species grew at 37° C.; i. e., very few species could be parasitic to warm-blooded animals. Few species were killed at 37° C., and species not killed grew normally when the medium was cooled to 20° C.

8. Incubation at low temperatures shows that growth in some species will begin at temperatures very close to the freezing point, but that only a few species will actually develop in cold-storage temperatures.

9. None of the species was found to grow in an atmosphere of carbon dioxid, but no species was killed by such atmosphere.

10. In most species failing to grow in a particular medium a change of concentration or the addition of a missing element will permit normal growth unless killed by osmotic pressure or definitely toxic agents.

KEYS TO CULTURAL IDENTIFICATION OF SPECIES.

Media: Prepare the following media—

1. 15 per cent gelatin[a] in distilled water.
2. 15 per cent gelatin in distilled water plus 3 per cent cane sugar.
3. Either bean or potato decoction plus 1.5 per cent agar.
4. Bean or potato agar plus 3 per cent cane sugar.

Litmus solution may be added if desired when cultures are made.

Prepare Petri dishes with 10 c. c. of each of the media used and allow them to cool. Inoculate two or more Petri dishes of each medium with spores of the species under examination. Incubate at 20° C. (the temperature of the working laboratory is usually

[a] In most of these studies the "gold-label" gelatin imported by Bausch & Lomb from Germany has been used. Culture of a species in a solution of gelatin in water has two uses in this paper—the detection of the production of enzyms capable of liquefying this medium, and the estimation of the ability of the species to grow in a medium free from carbohydrates. Neither of these tests seems to be vitiated by media made up at different times from materials of different origin. Such differences as are induced by the differences in the gelatin are quantitative, not qualitative.

satisfactory). Examine at intervals of three days or less, making observations with the naked eye from above and below, with the hand lens and with the compound microscope, using 16 mm. and 8 mm. or 6 mm. objectives to determine details of structure and fruit formation from growing colonies. A drop of litmus solution upon the margin of the colony will test acidity or alkalinity. Examine 1 and 2 for liquefaction, 2 and 4 for coremium and sclerotium formation. Sclerotium formation will be found to call for continued examination for at least two weeks.

Two separate keys are presented in the following pages: (1) A general key to all the forms discussed in this paper, based upon cultures in the media referred to above, and (2) a key to those species for which presence upon a particular substratum establishes a presumption of identity.

The species of most economic importance are found in key 2. Key 1, however faulty, is an endeavor to analyze the data from many series of comparative cultures in such a way as to simplify the identification of the forms included as far as possible. If the complexity of the data offered proves a barrier to identification it may be hoped that it will also deter those who fail to identify species clearly from using specific names in discussing work done with forms of this genus.

Key 1.—Analysis of Species in Cultures upon Gelatin and Agar.

A. Species fruiting typically by coremia (vertical and definite).
- *a.* Coremia long (3–15 mm.).
  - 1. Conidial masses strictly terminal, olive green, fragrant..*P. claviforme.*
  - 2. Upper third of coremia fertile, conidia green.....*P. duclauxi.*
- *aa.* Coremia small:
  - 1. Coremia definite, densely crowded, colony orange below, *P. granulatum.*
  - 2. Coremiform character indicated in cultures by clustering of conidiophores, definite coremia only in old cultures, becoming large and definite upon apples.....*P. expansum.*

AA. Species not (or rarely) producing coremia in culture.

B. Species constantly producing sclerotia or ascigerous masses.
- *b.* Producing ascigerous masses, yellow or reddish.....*P. luteum.*
- *bb.* Sclerotia appearing as white masses in old cultures.....*P. italicum.*[a]
- *bbb.* Sclerotia reddish or pink, globose or elliptical, 500μ or less in diameter.
- *c.* Conidial fructification a column:
  - 1. Column dense, long, sclerotia partially buried in substratum, *P.* No. 30.
  - 2. Column formed of loose chains, sclerotia numerous, exposed, *P.* No. 29.
- *cc.* Conidial fructification of divergent chains:
  - 1. Rapid liquefier, spores globose, 2.5–3μ.....*P.* No. 31.
  - 2. Slow liquefier, spores elliptical, 3.5–4×2.5–3μ.....*P.* No. 32.

[a] In its earlier development *P. italicum* Wehmer will be usually thrown under the head *kk*, on account of its habit of growth.

BB. Sclerotia not (or rarely) produced (under special conditions).
(Use gelatin cultures (1) and (2), compare agar cultures).
C. Rapid liquefiers (abundant liquid in 5 to 12 days).
D. With definite, strong ammoniacal odor:
1. Yellowish brown-avellaneous spores rough.....*P. brevicaule.*
2. White or cream, spores rough.....*P. brevicaule*, var. *album*.
3. White or cream, spores smooth.....*P. brevicaule*, var. *glabrum.*
DD. Without ammoniacal odor.
E. With yellow coloration of liquefied gelatin (*not of mycelium* in reverse).
1. Colonies small, conidiophores 100–150μ in length.....*P. citrinum.*
2. Colonies broadly spreading, conidiophores 250–300μ..*P. chrysogenum.*
EE. Without yellow color in liquefied gelatin (or slight traces only).
e. Colonies white to pink or salmon.....*P. roseum.*
ee. Colonies some shade of green.
f. Colonies floccose, margin spreading by stolons.....*P. stoloniferum.*
ff. Colonies velvety-surface growth of fruiting hyphæ only.
g. Conidiophores very short (100–200μ):
1.....*P.* No. 12.
2.....*P.* No. 37.
gg. Conidiophores longer (200–400μ):
1. Conidiophores variously branched, reverse always colorless..*P.* No. 24.
2. Conidiophores each with a verticil of branches—each branch bearing a columnar fructification—reverse and medium darkened in sugar media.....*P. atramentosum.*
CC. Liquefaction of gelatin none or slower than 10–12 days, or only partial.
G. Colonies never green.
h. Colonies yellowish brown, spores elliptical.....*P. divaricatum.*
hh. Colonies white to lilac, slow liquefier, 14–16 days.....*P. lilacinum.*
hhh. Colonies floccose white or creamy:
1. Conidiophores long, typical penicillate branching, *P. camemberti*, var. *rogeri.*
2. Conidial chains borne upon short branches of floccose hyphæ, *P.* No. 33.
GG. Colonies some shade of green.
H. Surface with hyphæ definitely in ropes or trailing, bearing numerous conidiophores as short branches distinctly traceable to their origin in such hyphæ.
i. Colonies usually red below and reddening the substratum.
1. Fruiting areas dark green.....*P. funiculosum.*
2. Fruiting areas mixed yellow and green.....*P. pinophilum.*
ii. Colonies not producing red color:
1. Colonies gray rarely greenish, very loose floccose.....*P. intricatum.*
2. Colonies green, conidial chains in simple compact columns..*P.* No. 28.
3. Colonies gray to green, hyphæ scattered, creeping.....*P. decumbens.*

HH. Surface hyphæ not in well-defined ropes, nor trailing.

j. Surface hyphæ woven floccose, course of hyphæ not traceable.

1. Gray-green, long conidiophores, no odor.....*P. camemberti.*
2. Gray-green, shorter conidiophores, strong odor.....*P. biforme.*

jj. Surface growth at margin simple conidiophores, in older parts both floccose hyphæ and conidiophores.

1. Gray-greenish, branching of conidiophore rather loose, odor none or slight.....*P.* No. 22.
2. Green, conidial fructifications rather compact, odor definite, "moldy".....*P. commune.*
   N. B.—There are probably a number of races in this group.

jjj. Fruiting surface velvety—of simple conidiophores or conidiophores borne so close to surface of substratum as to appear simple:

k. Conidial mass a dense column of conidial chains.

1. Column from a single verticil of basidia.....*P. spinulosum.*
2. Column from a verticil of brachlets with verticillate cells and chains.....*P. rubrum.*

kk. Elements of conidial fructification not in a column.

l. Conidia smooth.

1. Green, broadly spreading, ripe conidia globose, 4–5μ....*P. roqueforti.*
2. Green, less spreading, conidia elliptical, medium, commonly purpled.....*P. purpurogenum.*
3. Gray or olive green, conidia, 5–7 by 3–5μ.....*P. digitatum.*

ll. Conidia delicate rugulose.....*P. rugulosum.*

Key 2.—Species Determinable from Substrata.

(In these species the substratum establishes a presumption of identity.)

Cheese (Camembert and Brie):

1. Floccose, white unchangeable, no odor.....*P. camemberti* var. *rogeri.*
2. Floccose, white to gray-green, no odor.....*P. camemberti.*
3. Powdery, yellowish white, spores smooth, ammoniacal odor, *P. brevicaule* var. *glabrum.*
4. Powdery, yellowish white, spores tuberculate, ammoniacal odor, *P. brevicaule* var. *album.*
5. Forming yellowish-brown areas, spores rough, ammoniacal odor..*P. brevicaule.*

Cheese (Roquefort):

1. Green streaks inside the cheese.....*P. roqueforti.*

Citrus fruits:

1. Colonies of mold, blue-green.....*P. italicum.*
2. Colonies of mold, olive-green.....*P. digitatum=olivaceum.*

Pomaceous fruits (apples, pears, etc.):

1. Blue-green colonies finally producing coremia.....*P. expansum.*

Polyporaceæ (Boleti, Polypori, etc.):

1. Colonies green (yellowish green) spreading by stolons.....*P. stoloniferum.*

Wood (pine):

Producing orange to red stains in pine wood.....*P. pinophilum.*

TABULAR STATEMENTS.

TABLE 1.—*Tabulation of salient characters of species of Penicillium.*

[*Terms used.*—Colony characters: "Surface" growths are "strict" when conidiophores only rise above the substratum; "floccose" when condiophores arise as branches of aerial hyphæ; "mixed" when they arise both ways. "Margin" refers to the edge of the growing colony, which is usually either broadly spreading (broad) or restricted in habit (narrow). The lengths of the conidiophores are given in micromillimeters, and the limits given are those usual in peptone milk-sugar gelatin or in bean agar, observed within ten days. The conidial fructifications are designated as "loose" when the chains are more or less divergent, as "columns" when the chains are closely parallel or actually adherent into solid masses. In measurements of conidia the approximate limits of variation in diameter are given for globose conidia or the variations of the long and short axes of elliptical forms. In some cases both forms are noted.]

No.	Name.	Colony.		Length of conidiophore.			Conidial fructification.		Size of conidia.
		Surface.	Margin.	Minimum.	Average.	Maximum.	Form.	Length.	
				Microns.	*Microns.*	*Microns.*		*Microns.*	*Microns.*
1	*P. pinophilum*	Floccose	Broad	100		200	Loose	Up to 120	2 by 3-3.6
2	*P. brevicaule*	Mixed	do	10		30	do	Up to 150	6.5-7.5 by 7.5-9
3	*P. brevicaule* var. *glabrum*	do	do	10		30	do		7-8 by 8-10
4	*P. brevicaule* var. *album*	do	do	15		40	do		9-10
5	*P. camemberti*	Floccose	do	300		800	do	100-175	4.5-5.5
6	*P. camemberti* var. *rogeri*	do	do	100		800	do	70-90	4.5-5.5
7	*P. chrysogenum*	Coremia	do	Coremia 5,000-15,000.			Columns	Up to 1,000-2,000	4.2-4.6 by 3-3.3
8	*P. lilacinum*	Floccose	do	Very short, mostly.			Loose	Up to 100	2.5-3 by 2
9	*P. granulatum*	Coremia	do	Very short or very long.			do	100-200	2.5-3 by 3-3.5, or 3
10	*P. italicum*	Strict	do	100	270	600	do	Up to 500	2.3 by 3-5
11	*P. luteum*	Floccose	do	20	70-140	100	do	Up to 80	2-4 by 2-3
12		Strict	Narrow	40	65	125		40-80	3-3.5
13		Mixed	Broad	100		300	Loose		
14	*P. expansum*	do	do	50	100-200	1,000	do	130-1,000	2 by 3-3, or 3-3.4
15	*P. citrinum*	Strict	Narrow			150	Columns	50-150	2-4-3
16	*P. depilitum*	do	Broad	30		100+	Loose	Up to 100	4-7 by 6-8
17	*P. purpurogenum*	do. (?)	Narrow (?)	Variable with the media.					2-2.5 by 3.5-3.8
18	*P. roqueforti*	Strict	Broad	200		500	Loose columns	90-140	4-5
19	*P. roseum*	Floccose	do	45		125	Masses	Up to 140	5-7 by 3-5
20	*P. duclauxi*	Two forms	Two forms	10-70 or coremia.			Loose	100-140	3.6-4 by 2-2.5
21	*P. rubrum*	Mixed		15		30	Columns	100-200	2.5 by 3-3
22		Floccose	Broad	100	300	600	Loose	80-180	3-4
23	*P. commune*	do	do	100	500	700	do		
24		Strict (?)	Narrow	100	200	400		Up to 200	3.3-4
25	*P. chrysogenum*	do	Broad		300		Loose	100-200	3-4
27	*P. stolonifferum*	Floccose	Narrow	100 or less		600	do	Up to 170	28-3.4
28		do	Broad	Very short or very long.			Columns	Up to 500	2-2.4 by 3-3.3, or 2-3
29		do	do	80	200	400	Loose columns	Up to 400	3-3.6 by 2.3-2.8
30			do	230	300	500	Columns	Up to 500	2-5-3
32			do	200		500	Loose		3.5-4 by 2-3
33		Floccose	do				do		

34	*P. divaricatum*	Strict	do				do		5–7 by 2.5–3
36	*P. decumbens*	Floccose	Narrow	100		200	Columns	Up to 100	
37		Strict	do		100		do	Up to 600	2.5–3
38	*P. atramentosum*	do	do	240	300	400	do	100–200	3.5–4 by 2.5–3
39	*P. biforme*	Two forms	Broad	60–80		180–200	Loose	100, rarely 240	3.5–4.3
42	*P. funiculosum*	Floccose	do	20	80	100		125–160	3–4 by 2–3
45	*P. spinulosum*	Mixed	do	150–300 or more.			Columns	Up to 300+	3.2–3.5 by 3.6–4
46	*P. rugulosum*	Strict	Narrow	100		200	Loose	100–150	3.4–3.8 by 2.5–3
49	*P. intricatum*	Floccose	Broad	30		70	Loosely columnar	50–140	2.5–3
51		Strict (?)	Narrow	30		200	do	Up to 300	3

TABLE 2.—*Gelatin and color reactions.*

Species.		15 per cent gelatin in water.		Color.[a]		
No.	Name.	Liquefaction.	Litmus.	In conidial mass.	In medium.	Reverse of colony.
1	*pinophilum*	Very slow, 6 weeks.	Acid	Green	Yellow to red	Red.
2	*brevicaule*	Rapid, 5 to 6 days.	Alkaline	Drab to chocolate.	None	None.
3	var. *glabrum*	do	do	White or cream	do	Do.
4	var. *album*	do	do	do	do	Do.
5	*camemberti*	None or very slow.	do	Gray-green	do	Cream.
6	var. *rogeri*	do	do	White	do	Do.
7	*claviforme*	Slow	Neutral	Olive	Brownish	Brownish.
8	*lilacinum*	14 to 16 days	Alkaline	White or lilac	None	None.
9	*granulatum*	None	do	Yellowish green.	Yellow to orange.	Yellow to orange.
10	*italicum*	None or slow	Faint alkaline.	Bluish green	None	Brownish areas.
11	*luteum*	None	Acid	Green, small areas.	None or ?	Yellow to orange.
12		6 days	Alkaline	Pale blue-green.	None	None.
13		None in 15 days.	do	Deep green	do	Do.
14	*expansum*	Partial, 14 days	do	Green to gray-green.	do	None or brownish.
15	*citrinum*	Rapid, 4 to 6 days.	do	Green	Lemon yellow.	None or yellowish.
16	*digitatum*	None in 15 days.		Olive	None	Brownish.
17	*purpurogenum*	do		Green	Red or none	Yellow to red or none.
18	*roqueforti*	do	Alkaline	do	None	None.
19	*roseum*	Rapid, 8 days	do	White or salmon.	do	White or cream.
20	*duclauxi*	None in 15 days.	See color	Olive or green.	Yellow, acid. Red, alkaline.	Yellow, acid. Red, alkaline.
21	*rubrum*	(?)	(?)	Green or various.	(?)	Reddish.
22		Partial, 15 to 20 days.	Slow alkaline	Gray-green	None	White or cream.
23	*commune*	do	Alkaline	Green	do	Do.
24		Rapid 8 to 10 days	do	Deep green	do	Do.
25	cf. 26	do	do	Green	Yellow in certain media.	White or yellowish.
26	*chrysogenum*	do	do	do	None or golden	None or yellowish.
27	*stoloniferum*	Rapid, 6 to 8 days.	do	Yellowish green.	None or ?	Cream or yellowish.
28		Very slow, 20+ days.	Acid	Gray-green to green.	Yellow! violet!	None or yellowish.
29		Rapid	Alkaline	White, some green.	None or slight.	Do.
30		Very slow	Acid	Green	None	None.
31		Rapid	Alkaline	Gray or greenish.	None or yellowish.	None or yellowish.
32		Partial, slow, 15+ days.	Neutral?	Gray-green	None or reddish.	Yellowish to salmon.
33		None	Acid	White or cream.	None	Cream.
34	*divaricatum*	do	do	Yellowish brown.	do	None.
35	cf. 26	Rapid	Alkaline			
36	*decumbens*	None or ?	Neutral?	Gray or green.	None	White.
37		Rapid, 6 to 7 days.	Alkaline	Gray to blue-green.	do	Do.
38	*atramentosum*	Rapid, 6 to 10 days	do	Deep green	None or brown	None or brown.
39	*biforme*	None in 15 days.	do	Gray-green	None	Cream.
40		do	do	Green	do	Do
41	=19	Rapid	do	(?)	(?)	(?)
42	*funiculosum*	None	Acid	Green	Red or none	Red or none.
43		None or ?	Neutral?	Light olive	Red	Orange to red.
44	cf. 26	Rapid	Alkaline	(?)	(?)	
45	*spinulosum*	Partial, slow	Acid	Green	None	Cream or violet.
46	*rugulosum*	None or ?	Neutral?	do	None or yellowish.	Yellow to orange red.
47		Rapid	Alkaline	(?)	(?)	
49	*intricatum*	None	do	Gray to greenish.	None	White or sulphur.
51		None or ? slow	do	Yellowish green.	do	Uncolored.
54		Partial and slow.	do	(?)	(?)	
56	=38	Rapid	do	(?)	(?)	

[a] "None" means the absence of a definite color, varying from hyaline to cream at times.

TABLE 3.—*Comparative cultures.*

No.	Cohn's solution.	Raulin's fluid.	Milk + $CaCl_2$, curdling.	Milk.		Natural substratum.	Data at 37° C.
				Rate of digestion.	Coloration.		
1	Germinated	.....	Slow, 15 days.	Very slow	Red	Wood staining.	Grew best.
2	Slow, but typical.	Germinated	10 days	Rapid	None	Soft cheese	Grew well.
3	Germinated only.	do	13 days	do	do	do	No growth.
4	do	do	8 days	do	do	do	Do.
5	Fair, white	Very heavy	9 days	Slow	do	Camembert cheese.	Do.
6	No growth	do	do	do	do	do	Do.
7	Slight	Typical	do	do	Reddish brown.	.....	Do.
8	Weak, typical.	.....	None	do	None	.....	Best growth.
9	Germinated only.	Typical	do	do	Slowly red	.....	No growth.
10	do	do	10 days	do	Yellowish	Citrus fruits	Killed.
11	do	Slow, yellow.	Very slow growth in milk.			.....	Best growth.
12	Slight	Slow, typical.	7 days	Rapid	None	.....	Killed.
13	Small growth	Good	10 days	Slow	do	.....	No growth.
14	Weak	Typical	8 days	do	Yellowish brown.	Apples	Do.
15	Good, no yellow.	Good, some yellow.	do	Rapid	Slight yellow.	.....	Slight growth.
16	Germinated	Some growth	None; poor growth in milk.			Oranges	Killed.
17	do	Slow growth.	13 days	Slow	Slight red	.....	Grew well.
18	Slight growth	Richly typical.	8 days	Rapid	None, or evanescent greenish.	Roquefort cheese.	None.
19	do	.....	Rapid	do	None	.....	Killed.
20	Germinated	Typical	9 days	Slow	Yellow to red.	.....	No growth.
21	.....	.....	13 days	do	None	.....	
22	Fair growth	.....	8 days	do	do	.....	Do.
23	Small colonies.	Typical	7 days	do	do	.....	Do.
24	Slowly typical.	Slow, typical	8 days	Rapid	do	.....	Do.
25	Weak	.....	do	do	Pale yellow	.....	Slow growth.
26	Not typical	Typical	7 days	do	Golden yellow.	.....	Good growth.
27	Typical	Slowly typical.	do	do	Slight, if any	Fleshy fungi.	Killed.
28	Weak growth	Typical	8 to 9 days	Slow	Pale yellow	.....	Do.
29	.....	do	9 days	do	None	.....	
30	Germinated	.....	do	do	do	.....	No growth.
31	Slowly typical.	Not typical	do	Rapid	do	.....	Do.
32	Small colonies.	Good growth	Very slow	Slow	do	.....	Grew best.
33	No growth	Typical	From top down.	Slight	do	.....	Killed.
34	Germinated	do	Slow	Little or none.	do	.....	Grew best.
35	.....	.....	9 days	Medium rapid.	Yellow	.....	
36	Germinated	Richly typical.	Very slow, 4 weeks.	Little or none.	None	.....	Slight growth.
37	Weak growth	Typical	.....	Fairly rapid.	do	.....	Killed.
38	Germinated	Weak growth	9 days	Rapid	Reddish brown.	.....	No growth.
39	Half typical	.....	8 days	Rather slow	None	.....	Do.
40	do	.....	do	do	do	.....	Do.
41	.....	.....	9 days	do	do	.....	
42	Germinated	Typical	Very slow	Little	Red where touched.	.....	Grew well.
43	No growth	.....	9 days	Slow, slight	Not colored	.....	Do.
44	.....	.....	7 days	Rapid	Golden yellow.	.....	
45	Germinated	Typical	Very slow	Slow	None	.....	Grew best.
46	.....	.....	9 days	Slight	do	.....	No growth.
47	.....	.....	8 days	Slow	do	.....	
49	Weak growth	.....	Slow, 3 weeks.	Very slow	do	.....	Grew best.
51	Small colonies.	.....	None, 4 weeks.	do	do	.....	No growth.
54	.....	.....	11 days	Slow	do	.....	
56	.....	.....	.....	.....	.....	.....	

TABLE 4.—*Comparative cultures in synthetic fluid (Dox's).*

No.	Carbon supplied as—					
	Cane sugar.			Lactose.		0.9 per cent lactic acid.
	1 to 20 per cent.	60 per cent.	75 per cent.	Growth.	Litmus.	
1	Typical	No growth		Not typical	No effect	
2	Slowly typical	Germinated only.	Slight growth	Slow, typical	do	Germinated.
3	do	Slight	do	do	do	
4	do	do	do	do	do	
5	Typical	Slowly typical	Slow	Typical	Acid	Typical, alkaline.
6	do	do	Slight	do	do	Do.
7	do	No coremia but acid.		Very weak growth.	No effect	Germinated.
8	do	Slight	No growth	Germinated only.	do	Slight.
9	do	Not typical	Germinated	Slow, weak	do	Not typical.
10	do	Slow, typical	No growth	Slight	Acid	Slight.
11	do	No growth	Germinated	do	No effect	Do.
12	do	Good, acid	Slow	Weak, typical	do	Weak.
13	do	do	do	Slow, typical	do	Good.
14	do	do	Not typical	do	do	Do.
15	do	Weak, no yellow.	Weak, half typical.	Slow growth	do	Slight.
16	Not typical	Germinated only.	No growth	Germinated	do	Germinated.
17	Typical	do	do	do	do	Slow.
18	do	Not typical	Weak	Weak	do	Weak.
19		No growth	No growth	Slight	do	
20	Not typical	Germinated only.	do	Germinated	do	Good, acid.
21			do	do	do	
22	Typical	Slowly good	Very poor	Rich growth	Acid	
23	do	do	do	do	do	Good.
24	do	Weak	Germinated, small.	Small growth	No effect	Do.
25	do	Good	Good	Typical	Acid	Typical.
26	do	do	do	Slowly typical	do	
27	do	do	do	Weak	No effect	Small colonies.
28	do	do	Fair	Typical	Acid	Not typical.
29			No growth	Slight	No effect	
30	Typical	Green, no sclerotia.		Slowly typical	Acid	Weak.
31	Not typical	do	Slight	Slight	No effect	No growth.
32	Typical	Weak	No growth	do	do	Good.
33	do	Germinated only.	do	do	do	
34	do	Slight	do	do	do	Fair, not typical.
35	do	Good	Good growth	Typical	Acid	
36	do	Very weak	Pinhead colonies.	Slight	No effect	Do.
37	do	Slowly typical	Half typical	Slowly growing	do	Small colonies.
38	do	do	Very weak	Slowly typical	do	No growth.
39	do	do	Slight	Typical	Acid	Typical, alkaline.
40	do	do	do	do	do	
41	Slowly typical	Weak	No growth	Slight	No effect	
42	Typical	do	do	do	do	Little growth.
43	do	do	do	do	do	Do.
44	do	Slowly typical	Good growth	Typical	Acid	
45	do	do	Pinhead colonies.	Slight	No effect	
46	do	Fair, not typical.	Germinated	do	do	
47	do	Typical	Weak	Good	Acid	
49	do	Weak	No growth	do	No effect	
51	do	Very small colonies.	Pinhead colonies.	Germinated	do	
54	do	Typical	Good	Slight	do	
56	do	Slowly typical	Weak	Slow, typical	do	

TABLE 4.—*Comparative cultures in synthetic fluid (Dox's)*—Continued.

No.	Carbon supplied as—				
	1.5 per cent lactose.	1 per cent glucose.	1 per cent glycerin.	Butterfat.	
				Growth.	Color in medium.
1	Good, good	Fair, good	Slight	Typical, slow	Yellow to red.
2	Fair, alkaline	Fair, alkaline	Germinated only	Slow	
3	do	do	do	Fair	
4			Slight growth	do	
5	Typical	Typical	Slow, typical	Slow, typical	None.
6	do	do	do	do	Do.
7	Good	Good, good	None	do	Tinged.
8	Fair	Fair, alkaline	Slight	do	Tinged brownish.
9	Good, good	Typical	do	do	Yellowish.
10	Very scanty	Fair, good	No growth	do	None.
11	Weak	Good, good	Germinated	Slight	Do.
12	Good, alkaline	Good, alkaline	Some not typical	Weak	Do.
13	do	do	Good	Good	Do.
14	do	do	Slow	do	Do.
15	Small colonies	do	Small colonies	do	Lemon yellow.
16	Germinated	Slight	Germinated	None	
17	Small colonies	Slight, good	No growth	Slow	Slowly red.
18	Weak	Good, alkaline	Weak	Good	None.
19	Slight		Slight	Slowly typical	Do.
20	Weak	Weak	do	do	Yellowish.
21					
22	Good	Typical	Typical	Typical	None.
23	do		do	do	Do.
24	Small	do	Weak	do	Do.
25	Typical	do	do		
26					
27	Typical	Typical	Fair colonies		
28	Slow, typical		do	Typical	Reddish brown.
29				Slow	None.
30	Fair	Typical	Very weak	Very slow	Do.
31	Typical	do	do	Very slow, typical	Do.
32	Slowly typical	do	Very small colonies.	do	Do.
33	Small growth	do	Slowly typical	Slowly typical	Slightly rusty.
34	do	Slow	Germinated	Weak	None.
35					
36	Small colonies	Small growth	Germinated	Weak	Do.
37	do	Typical	Slight	Slow	Do.
38	Slowly typical	do	Germinated	Typical	Do.
39	Typical	do	Typical	do	Do.
40	do	do	do	do	Do.
41					
42	Little growth	Little growth	Germinated	Coremiform colonies.	Do.
43	do	do	do	Slow, typical	Yellowish.
44					
45	Typical		Half typical	Typical	None.
46	Good	Good	Slow	Slowly typical	Slight yellowish.
47					
48	Typical	Typical	Slow	Fair	None.
49	Half typical	do	Slowly typical	Small, typical	Do.

TABLE 5.—*Decimal summary of comparative cultures in synthetic fluid (Dox's).*

Species.		Carbon supplied as—												
		Cane sugar.				Lactose.								
		Up to 20 per cent.		60 per cent. growth.	75 per cent. growth.									
No.	Name.	Growth.	Acid.			Growth.	Acid.	Levulose, growth.	Glycerin, growth.	Galactose, growth.	Lactic acid, growth.	Raulin's fluid, growth.	Cohn's solution, growth.	Characteristic odor.
1	*pinophilum*	1.0	1.0	0.1	0.0	0.0	0	1.0	0.2	0.8	.....	.....	0.1	0
2	*brevicaule*	−1.0	0.0	0.1	0.1	−1.0	0	0.8	0.1	0.8	0.1	0.1	−1.0	1
3	var. *glabrum*	−1.0	0.0	0.1	0.1	−1.0	0	.....	0.1	.....	.....	0.1	0.1	1
4	var. *album*	−1.0	0.0	0.1	0.1	−1.0	0	.....	0.3	.....	.....	0.1	0.1	1
5	*camemberti*	1.0	1.0	−1.0	0.5	1.0	1	1.0	−1.0	1.0	1.0	+1.0	0.8	0
6	var. *rogeri*	1.0	1.0	−1.0	0.5	1.0	1	1.0	−1.0	1.0	1.0	+1.0	0.0	0
7	*claviforme*	1.0	1.0	0.2	0.2	0.2	0	0.4	0.3	0.9	0.1	1.0	0.2	1
8	*lilacinum*	1.0	(?)	0.1	0.0	0.1	0	0.4	0.2	0.8	0.3	.....	−0.7	0
9	*granulatum*	1.0	1.0	0.3	0.1	0.5	0	0.5	0.4	1.0	0.6	1.0	0.1	0
10	*italicum*	1.0	0.0	−1.0	0.0	0.3	?1	0.5	0.3	0.9	0.3	1.0	0.1	1
11	*luteum*	1.0	0.0	0.1	0.1	0.2	0	0.2	0.1	1.0	0.3	−0.7	0.1	0
12	.....	1.0	1.0	0.1	0.6	0.7	0	1.0	0.4	1.0	0.4	−1.0	0.2	0
13	.....	1.0	1.0	1.0	0.8	−1.0	0	1.0	−1.0	1.0	0.8	1.0	0.4	1
14	*expansum*	1.0	1.0	1.0	0.4	−1.0	(?)	1.0	−1.0	1.0	.....	1.0	0.5	1
15	*citrinum*	1.0	1.0	0.5	0.5	−0.6	0	0.6	0.6	1.0	0.4	1.0	0.7	0
16	*digitatum*	(?)	(?)	0.1	0.0	0.1	(?)	0.1	0.1	0.4	0.1	0.4	0.1	1
17	*purpurogenum*	1.0	0.0	0.1	0.0	0.1	0	0.3	0.0	0.4	0.6	0.6	0.1	0
18	*roqueforti*	1.0	0.0	0.3	0.2	0.5	0	0.5	0.4	0.8	0.4	1.0	0.2	0
19	*roseum*	−1.0	0.0	0.0	0.0	0.2	0	0.2	0.2	.....	.....	.....	0.2	0
20	*duclauxi*	1.0	(?)	0.1	0.0	0.1	0	0.2	0.2	0.3	0.8	1.0	0.1	(?)
21	*rubrum*	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
22	.....	1.0	1.0	−1.0	0.3	−1.0	1	1.0	−1.0	1.0	.....	.....	0.8	(?)
23	*commune*	1.0	1.0	−1.0	0.6	−1.0	1	1.0	−1.0	1.0	1.0	1.0	0.5	1
24	.....	1.0	1.0	0.4	0.3	0.6	0	0.6	0.5	1.0	1.0	−1.0	−1.0	0
25	.....	1.0	1.0	0.7	0.7	1.0	1	1.0	0.7	1.0	1.0	.....	0.5	(?)
26	*chrysogenum*	1.0	1.0	0.7	0.7	−1.0	1	.....	0.7	.....	.....	1.0	0.5	0
27	*stoloniferum*	1.0	1.0	1.0	−1.0	0.6	0	1.0	0.9	1.0	0.5	−1.0	1.0	0
28	.....	1.0	1.0	1.0	−1.0	1.0	1	−1.0	0.9	.....	0.5	1.0	0.6	(?)
29	.....	.....	.....	0.0	0.2	.....	.....	.....	.....	.....	.....	1.0	.....	0
30	.....	1.0	1.0	0.4	.....	−1.0	1	1.0	0.2	1.0	0.4	.....	0.1	0
31	.....	1.0	1.0	0.5	0.2	0.2	0	1.0	0.2	1.0	0.0	0.5	−1.0	0
32	.....	1.0	1.0	0.3	0.0	0.2	0	−1.0	0.3	1.0	1.0	0.8	0.6	0
33	.....	1.0	(?)	0.1	0.0	0.2	(?)	0.3	−1.0	1.0	.....	1.0	0.0	0
34	*divaricatum*	1.0	1.0	0.1	0.0	0.2	0	0.3	0.1	0.4	0.7	1.0	0.1	0
35	cf. 26	1.0	1.0	1.0	0.7	1.0	1	.....	.....	.....	.....	.....	.....	.....
36	*decumbens*	1.0	1.0	0.2	0.5	0.2	0	0.3	0.1	0.4	0.7	1.0	0.1	1
37	.....	1.0	1.0	0.5	0.8	0.5	0	0.5	0.5	1.0	0.7	1.0	0.3	0
38	*atramentosum*	1.0	1.0	0.7	0.3	0.9	0	0.9	0.1	1.0	0.0	0.5	0.1	0
39	*biforme*	1.0	1.0	−0.9	0.4	1.0	1	1.0	−1.0	1.0	1.0	.....	0.7	1
40	.....	1.0	1.0	−0.9	0.4	1.0	1	1.0	0.8	1.0	.....	.....	0.7	1
42	*funiculosum*	1.0	1.0	0.1	0.0	0.2	0	0.2	0.1	0.3	0.3	1.0	0.1	0
43	cf. 17	1.0	1.0	0.1	0.0	0.2	0	0.2	0.1	0.3	0.3	.....	0.0	0
44	cf. 26	1.0	1.0	0.7	0.8	1.0	1	.....	0.5	.....	.....	.....	.....	.....
45	*spinulosum*	1.0	1.0	−0.8	0.5	0.2	0	1.0	0.6	.....	.....	1.0	0.1	0
46	*rugulosum*	1.0	1.0	−0.6	0.1	0.2	0	1.0	−1.0	0.9	.....	.....	.....	0
49	*intricatum*	1.0	1.0	0.3	0.0	1.0	0	1.0	−1.0	1.0	....	.....	0.3	0
51	.....	1.0	1.0	−1.0	0.8	0.2	0	.....	0.1	1.0	.....	.....	.....	.....

Explanation of decimals: 1.0 denotes normal development, or present; −1.0, slowly typical; 0.1, germination of spores only; decimals from 0.1 to 1.0 denote estimated amount of development between mere germination and typical development; +1.0 denotes very rich growth.

TABLE 6.—*Incubation experiments.*

Species.		20° C.	Six days at 37° C.			Ice thermostat.															
						Compartment 1, 0.5–2° C.				Compartment 2, 3.2–6° C., av. 4° C.				Compartment 3, 6–10° C., av. 7°+ C.				Compartment 5, 7–10.5° C., av. 8.7° C.			
						Growth, periods in days.				Growth, periods in days.				Growth, periods in days.				Growth, periods in days.			
No.	Name.	Growth.	Growth.	Killed.		7.	15.	23.	29.	7.	15.	23.	29.	7.	15.	23.	29.	7.	15.	23.	29.
1	*pinophilum*	1.0	+1.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1?	0.1?	0.1?	0.1	0.1?	0.1?	0.1?	0.1	0.3	0.5	0.6	
2	*brevicaule*	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	
3	var. *glabrum*	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.4	0.4	0.3	0.4	0.5	0.7	
4	var. *album*	1.0	0.0	0.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
5	*camemberti*	1.0	0.0	0.0	0.2	0.5	0.5	0.6	0.3	0.5	0.5	0.6	0.5	0.6	0.7	0.9	0.6	0.6	1.0	1.0	
6	var. *rogeri*	1.0	0.0	0.0	0.0	0.1	0.3	0.5	0.1	0.3	0.4	0.5	0.3	0.5	0.6	0.8	0.4	0.8	1.0	1.0	
7	*claviforme*	1.0	0.0	0.0	0.2	0.3	0.4	0.6	0.1	0.3	0.4	0.6	0.3	0.6	0.8	0.9	0.6	0.6	0.8	1.0	
8	*lilacinum*	1.0	+1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1?	0.1?	0.1	0.0	0.3	0.5	0.6	
9	*granulatum*	1.0	0.0	0.0	0.2	0.3	0.4	0.6	0.3	0.5	0.6	0.8	0.3	0.5	0.6	0.8	0.6	0.8	1.0	1.0	
10	*italicum*	1.0	0.0	1.0	0.1	0.8	0.9	0.9	0.3	0.7	0.8	0.9	0.5	0.8	0.9	1.0?	0.8	1.0	1.0	1.0	
11	*luteum*	1.0	+1.0	0.0	0.0	0.0	0.1?	0.1?	0.0	0.0	0.0	0.0	0.0	0.1?	0.1?	0.1?	0.1	0.3	0.3	0.5	
12	...	1.0	0.0	1.0	0.0	0.0	0.0	0.1?	0.0	0.1?	0.1?	0.1?	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.2	
13	...	1.0	0.0	0.0	0.2	0.5	0.6	0.8	0.3	0.5	0.7	0.9	0.4	0.7	0.9	1.0	0.6	1.0	1.0	1.0	
14	*expansum*	1.0	0.0	0.0	0.1	0.1	0.3	0.4	0.2	0.2	0.7	0.8	0.3	0.6	0.6	0.6	0.6	0.9	1.0	1.0	
15	*citrinum*	1.0	0.3	0.0	0.1	0.0	0.0	0.1?	0.1	0.1?	0.1?	0.1?	0.1	0.1	0.1	0.1	0.2	0.3	0.3	0.3	
16	*digitatum*	1.0	0.0	1.0	0.1	0.1	0.3	0.4	0.2	0.2	0.3	0.4	0.1	0.4	0.5	0.6	0.5	0.7	0.8	0.9	
17	*purpurogenum*	1.0	1.0	0.0	0.0	0.1?	0.1?	0.1?	0.1	0.1	0.1	0.1	0.0	0.1?	0.1?	0.1?	0.3	0.1	0.1	0.1	
18	*roqueforti*	1.0	0.0	0.0	0.2	0.3	0.7	0.8	0.25	0.5	0.7	0.9	0.4	0.7	1.0	1.0	0.6	1.0	1.0	1.0	
19	*roseum*	1.0	0.0	1.0	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1?	0.0	0.0	0.0	0.1	0.5	0.5	0.6	
20	*duclauxi*	1.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	...	0.0	0.1	0.1	0.1?	0.1?	0.1	0.1	0.3	0.4	0.4	
21	*rubrum*	1.0	...	...	0.0	0.0	0.0	0.0	...	...	...	...	0.0	0.0	0.0	0.0	...	...	...	...	
22	...	1.0	0.0	0.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
23	*commune*	1.0	0.0	0.0	0.3	0.6	0.6	0.8	0.4	0.6	0.7	0.9	0.6	0.9	1.0	1.0	0.6	0.8	1.0	1.0	
24	...	1.0	0.0	0.0	0.1	0.3	0.5	0.7	0.2	0.5	0.7	0.9	0.4	0.7	1.0	1.0	0.6	1.0	1.0	1.0	
25	=26	1.0	0.8	0.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
26	*chrysogenum*	1.0	1.0	0.0	0.1	0.1	0.3	0.6	0.4	0.5	0.6	0.8	0.4	0.6	0.8	1.0	0.6	1.0	1.0	1.0	
27	*stoloniferum*	1.0	0.0	1.0	0.2	0.1	0.1	0.3?	0.4	0.4	0.4	0.5	0.5	0.5	0.5	0.6	0.6	0.9	1.0	1.0	
28	...	1.0	0.0	1.0	0.0	0.0	0.1	0.1	0.0	0.1?	0.1	0.3	0.1	0.3	0.4	0.6	0.4	0.6	0.8	0.9	
29	...	1.0	...	...	0.0	0.0	0.1	0.3	0.0	0.1	0.2	0.6	0.1	0.5	0.5	0.6	0.4	0.7	0.8	0.9	
30	...	1.0	0.0	0.0	0.0	0.1	0.4	0.5	0.0	0.0?	(?)	(?)	0.0	0.4	0.7	0.8	0.0	0.7?	(?)	(?)	
31	...	1.0	0.0	0.0	0.2	0.5	0.6	0.6	0.4	0.5	0.7	0.8	0.5	0.8	1.0	1.0	0.6	0.7	0.8	0.9	
32	...	1.0	+1.0	0.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
33	...	1.0	0.0	1.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
34	*divaricatum*	1.0	+1.0	0.0	0.0	0.0	0.0	0.0	0.1?	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.2	0.3	0.3	
35	cf. 26	1.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
36	*decumbens*	1.0	0.2	0.0	0.0	...	...	...	0.0	0.1	0.2	0.2	0.1	0.3	0.4	0.4	0.2	0.7	0.7	0.8	
37	...	1.0	0.0	1.0	0.1?	0.1?	0.1?	0.1?	0.1	0.1?	0.1?	0.1?	...	...	...	...	0.1	0.3	0.3	0.3	
38	*atramentosum*	1.0	0.0	0.0	0.1	0.1	0.3	0.5	0.1	0.1	0.4	0.7	0.2	1.0	1.0	1.0	0.8	0.9	1.0	1.0	
39	*biforme*	1.0	0.0	0.0	0.4	0.4	0.7	0.9	0.4	0.2	0.5	0.8	0.5	0.6	0.7	0.9	0.6	0.9	1.0	1.0	
40	cf. 23	1.0	0.0	0.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
41	=19	1.0	0.0	1.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
42	*funiculosum*	1.0	1.0	0.0	0.0	0.0	0.0	0.1?	0.0	0.0	0.1	0.1	0.0	0.3	0.5	0.7	0.1	0.7	0.8	0.9	
43	cf. 17	1.0	1.0	0.0	0.0	0.0	0.0	0.1?	0.1	0.0	0.1?	0.1?	0.1	...	...	...	0.1	0.3	0.4	0.5	
44	cf. 26	1.0	−0.4	0.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
45	*spinulosum*	1.0	+1.0	0.0	0.0	1?	0.1	0.3	0.1	0.1	0.2	0.3	0.4	0.5	0.7	0.7	0.6	0.7	1.0	1.0	
46	*rugulosum*	1.0	0.0	0.0	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.3	0.4	0.5	0.5	0.6	0.5	0.7	0.8	1.0	
47	...	1.0	0.3	0.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
49	*intricatum*	1.0	+1.0	0.0	0.0	0.0	0.0	0.1?	...	0.0	0.0	0.0	0.1	0.3	0.3	0.3	0.1	0.3	0.6	0.6	
51	...	1.0	0.0	0.0	0.1	0.1	0.1	0.1	...	0.0	0.0	0.0	...	0.3	0.4	0.4	(?)	0.7?	0.8?	1.0?	
54	...	1.0	0.0	0.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
56	=38	1.0	0.0	0.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	

Explanation of decimals: 0.1 denotes germination of conidia only; decimals up to 0.7, growth without the formation of colored conidial areas; 0.7 to 1.0, colonies with colored conidia; 1.0 denotes typical colony. +1.0 denotes growth more rapid at 37° than at 20° C.

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